Rapid and precise thermal conditioning for a DNA analysis chip

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RAPID AND PRECISE THERMAL CONDITIONING FOR A DNA ANALYSIS CHIP

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SCHOOL OF MECHANICAL & AEROSPACE ENGINEERING
NANYANG TECHNOLOGICAL UNIVERSITY

2005

**Rapid and Precise Thermal
Conditioning for A DNA
Analysis Chip**

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School of Mechanical & Aerospace Engineering

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ABSTRACT

The development and applications of polymerase chain reaction (PCR) for DNA analysis are becoming increasingly important in molecular diagnostics and drug development processes. Many conventional PCR thermal cycling systems use glass or plastic based tubes with relatively big reaction volumes ($>10\mu\text{L}$) to conduct PCR. This resulted in a long time to reach thermal characteristics required for the PCR reaction and higher reagents consumption because of the larger reaction volume and imprecise temperature control involved in the conventional millimetre size PCR reaction tubes. With the applications of MEMS technologies, PCR microchips can be developed to achieve rapid thermal cycling and precise temperature control in the PCR process and the subsequent DNA melting analysis.

The present work aims to develop, analyse and fabricate a PCR microchip to improve the DNA amplification process to achieve a rapid PCR thermal cycling and a precision DNA melting analysis. The PCR chip consists of an array of reaction chambers, platinum thin film heaters and temperature sensors, and air convection channels for forced cooling. An analytical model was developed to investigate the thermal transport processes in the proposed PCR microchip. These models provide an in-depth understanding of the thermal characteristics of the chip during the PCR and the DNA melting process, and serve as an effective design tool in the preliminary design stage of the chip.

2-D and 3-D transient heat transfer numerical simulations using ANSYS software were conducted to assess and verify the performance of the proposed chip. The results indicated that a rapid thermal cycling and precise temperature control can be achieved on this microchip. Numerical investigations on effect of chamber characteristics during the PCR process and the DNA melting process were carried out to provide critical parameters for the chip design.

To experimentally demonstrate and investigate the rapid PCR and precision DNA melting analysis, a microchip with four reaction chambers was fabricated on a glass

substrate using MEMS techniques as an initial attempt for the DNA analysis microchip with a large number of reaction chamber arrays.

Experiments on chip thermal characteristics were carried out on the microfabricated chip and the results were compared with commercial PCR systems. The chip system shows superior thermal characteristics in PCR process and precise temperature control in DNA melting analysis. The experiment results show a good match with the numerical simulation results.

Molecular biology experiments were carried out to evaluate the performance of the micro-chip during PCR process and DNA melting curve analysis. Comparing with the results from commercial PCR machines, the chip system can successfully perform a rapid and precise real-time quantitative PCR, multiple PCR in multiple reaction wells in a single chip, and precise detection of melting temperature difference for DNAs with single base mismatch during DNA melting curve analysis.

Improvements on reaction chamber sealing and microfluidics design for sample loading and sealing were briefly described. The discussion on future work focused on fine-tuning the chip design to achieve high density PCR reactions.

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Chapter 1 Introduction

1.1 Background

In 1985, Randall Saiki et al. published a paper entitled “Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia” [1] in the journal *Science*, which described a method to detect single base pair changes in the beta-globin gene and became the first to use the polymerase chain reaction (PCR). During the PCR process, molecular replications take place when a double-stranded fragment is first thermally separated (or denatured) into two strands followed by polymerase action. This is an important step forward in modern molecular biology. Subsequently, Saiki et al. simplified the process of performing PCR in their 1988 *Science* paper [2], when they used a thermostable DNA polymerase from the organism *Thermus aquaticus* (Taq). In 1991, Higuchi et al. [3] presented the addition of ethidium bromide (EtBr) to PCR that would allow external monitoring of the reaction process easily. Today, PCR has become a powerful procedure used in the analysis of DNA sequences, which is rapidly replacing many other time-consuming and less sensitive techniques for the identification of biological species and pathogens in forensic, environmental, clinical and industrial samples.

With the development of micro-electro-mechanical-systems (MEMS) technology, miniaturization of PCR systems has become viable, which are able to sample, chemically process, and analyze target biomolecular. This essentially constitutes a “lab on a chip”. The miniaturization technology is called the micro PCR, which offers fast reaction, low reagent consumption (in nano or pico liter volume) and integration of heating/cooling, temperature sensing and detection devices on to the same chip microfabricated.

1.2 Current problems

In the development of PCR technology, many problems with conventional PCR

machines using tubes can be improved with the micro PCR. But still, some problems have not been solved.

Firstly, there is a lack of investigations on how to use heat transfer characteristics to achieve rapid and precise temperature control.

Secondly, the current research on micro PCR only focuses on chips with single PCR chamber. Very few investigations on PCR chamber array were carried out. Quanbo Zou etc [5] and Etsuo SHINOHARA etc [6] attempted to develop PCR chamber arrays, but they didn't investigate precise temperature control.

Thirdly, current micro PCR technologies lack active cooling specific to individual reaction chambers during thermal cycling. All micro PCR devices reduce the temperature by a common free or forced convection, which is not suitable for non-homogenous PCR scheme in a reaction chamber array.

Fourthly, few investigations of thermal cross-talk in PCR chamber array were carried out.

The last problem is that no on-chip DNA melting analysis was performed after PCR process and there is a lack of investigations on precise temperature control in DNA melting analysis.

1.3 Objectives

The problems mentioned in section 1.2 have become the bottlenecks of micro PCR technology development. The aim of this project is to develop and investigate a PCR microchip with the following features:

1. Rapid (single PCR cycle is less than 10 seconds) and precise (the PCR temperature accuracy is 0.1°C) PCR thermal cycling.
2. An array of PCR chambers (at least 4 chambers) on a single chip which can achieve more than one individual PCR reactions simultaneously with a different

thermal cycling profile in each PCR chamber.

3. Precise temperature control (the temperature resolution of DNA melting analysis is 0.001°C) during DNA detection.

1.4 Scope

The scope of this work in the two years contains the following seven portions. Chapter 1 presents an introduction of the entire project; Chapter 2 reviews the development of micro PCR technology and PCR microchip; Chapter 3 presents the methodology and design of a microchip. The numerical simulations of on-chip heat transfer and the chip fabrication processes are presented in Chapter 4 and 5 individually. In Chapter 6, the thermal and biologic experiments and results of PCR process and DNA melting curve analysis in chip PCR system are shown clearly. The current work is summarized in Chapter 7, as well as the future directions of the project.

1.5 Contribution

Novel contributions from this project are the following:

1. Design and fabrication of a microchip with rapid thermal cycling and uniform temperature in the PCR process and precise temperature scan control in the DNA melting analysis.
2. Design of a micro PCR chamber array with high throughput PCR capability.
3. Simulation and investigation of the effect of chamber characteristics on PCR speed and temperature uniformity, precise temperature control during DNA melting analysis and thermal cross-talk in PCR chamber array.
4. Optimization the microchip parameters based on both simulation and experiments, which can serve as a guideline for chip design.
5. Successful thermal experiments on PCR cycling in microfabricated chip.
6. Successful thermal experiments on precise temperature scan for DNA melting

analysis

7. Successful Bio-experiments on rapid PCR in the chip.
8. Successful Bio-experiments on multiple PCR in the chip.
9. Successful Bio-experiments on DNA melting curve analysis.

Chapter 2 LITERATURE REVIEW

2.1 Introduction

Although microfabrication technology in micro PCR chip is still in research stage, its future has been foreseen to be substantially and promising. This chapter attempts to give a review from conventional devices and methods to the latest development of miniaturizing PCR chips.

2.2 DNA analysis methods

Genetic information in humans stored in the cell chromosomes. Each chromosome consists of long, compactly packed, super-coiled linear polymer strands of deoxyribonucleic acid (DNA) [7]. The chromosome information is stored in a long string of DNA fragments grouped as genes, each expressing an identifiable function or characteristic of the organism. Each nucleotide consists of a base (B), a sugar linkage (S), and a phosphate bridge (P), as shown in Figure 2.1(a). The sugar linkage gives the nucleotide directionality with two distinct ends labelled 5' and 3'. There are four types of nucleotides corresponding to four different bases: adenine, guanine, cytosine, and thymine, commonly labelled A, G, C and T. Nucleotides can only be linked in a specific direction forming single strands of DNA as shown in Figure 2.1(b). Individual bases are hydrophobic, but strands of DNA are quite soluble in water due to the polar backbone. Single stranded DNA tends to attach (or hybridise) another strand of complementary base pairs (G-C and A-T) through weak hydrogen bonds and form a double strand (or duplex) as shown in Figure 2.1(c). Double-stranded DNA is more stable in water because the hydrophobic bases are hidden by hydrophilic backbones.

Figure 2.1: Structure of DNA. (a) DNA molecule, (b) single stranded DNA chain, (c) double stranded DNA with complementary base pair (bp). The two strands are joined by hydrogen bonds [8].

DNA assays take two general forms. In diagnostic applications, the assay detects the presence of a specific base pair fragment in a fingerprint pattern matching fashion. In sequencing applications, the assay yields actual base pair order. Sequencing assays inherently provide much more information than fingerprinting assays since test patterns are often not unique due to the presence of mutations.

Conventional genetic assay protocols consist of three main steps for the analysis of DNA and each of which may be composed of different procedures depending on specific application. The first step is sample preparation whose function is to provide suitable DNA sample for the following steps. The concentrations of the components in a DNA sample may affect the efficiency of reactions. The second step is DNA amplification that employs diverse chemical reactions to duplicate target DNA segments in order to increase concentration of target DNA segment to detectable level. The third step is detection. There are several different methods currently available to achieve function of detection, such as fluorescence method and electrophoretic method.

In the above three steps, the amplification is the most important process to increase speed of DNA analysis. The conventional amplification may cost several hours, but now it can be finished in one hour or even less. In addition to other DNA detection methods, the method DNA melting analysis is an efficient method to distinguish amplicons. A high resolution is required in more demanding research areas of genetics.

2.3 Polymerase Chain Reaction (PCR)

DNA sample are often presented at concentration levels that are too low for any direct detection. Therefore, a chemical amplification is used to increase the concentration of sample. The amplification basically consists of a set of reactions to duplicate a DNA molecule or fragment. Further amplification is hence obtained by repeating the procedure. [9]

Powerful enzymes are used for amplification, which assemble complementary strands of DNA from a single-strand fragment. The enzyme scans single strands of DNA from a specific location, captures matching nucleotides from solution and connects them to the single strand, assembling the complementary strand one base at a time like a zipper. [10]

Molecular replications take place when a double-stranded fragment is first thermally separated (or denatured) into two strands followed by polymerase action. This procedure is known as the polymerase chain reaction (PCR) invented by Kary Mullis [11]. A typical amplification uses high-temperature resistant polymerase enzyme, and adequate supply of nucleotides, and primers that determine the starting point of the replication. It seems that PCR is an uncomplicated technique. All that happens is that a short region of DNA molecule is copied many times by DNA polymerase enzymes. The length of the amplified fragment is controlled by cleaving the DNA ahead of time or by auxiliary primers.

The PCR amplification process involves a number of repeated temperature cycles (typically 20-40) resulting in an exponential increase of strands with sequence of interest between primer sites [12]. To begin amplification, the enzyme is added to the primed template DNA and incubated, so that it synthesizes new complementary strands (see Figure 2.2a). The mixture is then heated to 94°C, so that newly synthesized strands detach from the template (as shown in Figure 2.2b), and cooled, enabling more primers to hybridise at their respective positions, including positions on the newly synthesized strands. The enzyme now carries out a second round of

DNA synthesis (Figure 2.2c). The cycle of denaturation-hybridization-synthesis is repeated, usually 25-30 times, resulting in the eventual synthesis of several hundred million copies of the amplified DNA fragment, as seen in Figure 2.2d. At the end of the PCR experiment, a sample of the reaction mixture is usually analysed by agarose gel electrophoresis. Sufficient DNA has been produced for amplified fragment to be visible as a discrete band after staining with ethidium bromide (Figure 2.2e).

Figure 2.2: Principle of PCR [10]

2.4 Real time Quantitative PCR

Theoretically, there is a quantitative relationship between amount of starting target sequence and amount of PCR product at any given cycle. However in practice, the molecular amplification process is not straightforward as described in theory. The amplification eventually reaches a plateau, and it may not be exponential to begin with because of variations in reaction conditions or the presence of inhibitors. Hence, it might result the number of copies of molecular amplification to be inaccurate.

2.4.1 Fluorescence detection technology

Normally, there are two general methods for the present of amplified DNA fragments; one is fluorescence method and the other is electrophoretic method. Because of extraordinary sensitivity, high specificity, simplicity, and low cost, the fluorescence detection technology is usually selected.

Fluorescence is the molecular absorption of light energy at one wavelength and its nearly instantaneous re-emission at another, usually longer, wavelength. Some molecules fluoresce naturally and others can be modified to make fluorescent compounds. No two compounds have same fluorescence signature. So it is a powerful and widely accepted technique used for a variety of environmental, industrial, and biotechnology applications. [13, 14, 15]

2.4.1.1 Sybr-Green I (SG) method

SG is an intercalating fluorescent dye that binds to the minor groove of the DNA double helix [16]. The unbound dye exhibits little fluorescence in solution, but upon binding to double-stranded DNA the fluorescence is enhanced. This is utilized in real-time amplification. As DNA is amplified during an amplification reaction, the dye binds to amplified products and the fluorescent signal is increased as Figure 2.3. The increase fluorescence signal is analyzed against the background fluorescence level. It is an easy and effective approach to real-time detection, as it does not require the design of sequence specific probes and new primer sets [18]. Intercalating dyes

allow for the melting of amplification products at the end of a run. This is called DNA melt analysis [19] which will be described in section 2.6.

Figure 2.3 Principle of Real-time PCR with Sybr-Green I(SG) method (A) SG (S) will not bind to ssDNA (single-stranded DNA) and the intensity of fluorescent signal is low (B) SG (S) binds to dsDNA (double-stranded DNA); the fluorescent signal intensity (E) increases

2.4.1.2 TaqMan method

A Taqman probe is a single oligonucleotide dual labelled with a fluorescent reporting dye attached to the 5'-end and a quencher molecule attached to the 3'-end. Because the probes are only 15-25 base pairs long, the reporter dye and quencher are in close proximity to each other and little fluorescence is detected. During the cycling process, Taq DNA polymerase extends from each primer. The DNA polymerase has an exonuclease activity that cleaves the downstream probe as it extends. As the probe is degraded, the reporter dye is separated from the quencher, generating a sequence-specific fluorescent signal (Figure 2.4). As further PCR cycles proceed, the fluorescence increase is measured in real time [20]. Because the reporting dye is cleaved, melt curve analysis is not possible. Dual labelled probes offer higher specificity compared to intercalating dyes. Due to the increased specificity of dual labelled probes, this detection method works well for low copy number templates [21].

Figure 2.4 Principle of Real-time PCR with Taqman method [21]

2.4.1.3 Molecular Beacon method

Molecular Beacons (MB) probes are based on a hairpin structure design with a reporter fluorescent dye on one end and a quencher molecule on the other end [22] as shown in Figure 2.5. The hairpin structure causes the MB probe to fold when not hybridized. This brings the reporter and quencher molecules in close proximity with little to no fluorescence being emitted. However, when the MB hybridizes to the template, the hairpin structure is broken and the reporter dye is no longer quenched. At this point, the real-time instrument detects fluorescence. A DNA melt analysis is also possible with Molecular Beacons [23].

Figure 2.5: Principle of Real-time PCR with Molecular Beacon method. The hairpin structure causes the MB to fold when not hybridized, bringing quencher and fluorophore dyes in close proximity causing quenching of fluorescence. When hybridized, the fluorophore and quencher are separated resulting in increased fluorescence.

2.4.1.4 FRET method

FRET probes rely on transfer of energy from one fluorescent dye to another. Two separate sequence specific oligos are fluorescently labelled. The upstream probe has a donor molecule on the 3'-end and the downstream probe has an acceptor molecule on the 5'-end. The probes are designed so that they hybridize adjacently to each other on the target sequence and bring the donor and acceptor fluorophores in close proximity (Figure 2.6). Once the probes are hybridized, the donor and acceptor fluorescent molecules are in close proximity to one another. This allows for transfer of energy from the donor to the acceptor fluorophore, which emits a signal of a different wavelength. Either the decrease in the fluorescence of the donor or the increase in fluorescence of the acceptor can then be detected. Therefore, only when both probes are bound, fluorescence is detectable. FRET probes are extremely useful for genotyping, SNP detection, and other mutation detections [24].

Figure 2.6 Principle of Real-time PCR with FRET method. (a) Resonance energy (E) transfer is low when the probes are not hybridized. (b) Hybridization of the probes brings the donor (D) and acceptor (A) fluorophores into close proximity resulting in increased resonance energy transfer.

2.4.2 Standard curve quantitation

Real-time PCR is based on the detection and quantitation of a fluorescent reporter. Fluorescent signals increase in direct proportion to amount of PCR product. By recording the amount of fluorescence emission at each cycle, it is possible to monitor PCR reaction during exponential phase where the first significant increase in amount of PCR product correlates to initial amount of target template.

Time, temperature and fluorescence are the parameters that can be used to describe real-time fluorescence monitoring of PCR. Fluorescent data is collected at least once

during each cycle of amplification [26]. After the curve of fluorescence vs. time is established, data is normalized to account for differences in background fluorescence. Once normalization is complete, a threshold level can be set. This is a reflection of the amplification efficiency. The most important parameter for real time quantitation is CT, the cycle number to reach the threshold level, which has an exponential relationship with DNA copy number. With CT and corresponding concentration collected, a typical standard curve can be generated, from which the absolute copy number of unknown samples can be determined (Figure 2.7) [26, 88].

Figure 2.7: Determination of the absolute copy number of an unknown sample using a standard curve

2.5 Temperature control of PCR thermal cycling

The theoretical DNA amplification factor is 2^n , where n is defined as the number of PCR cycles performed [27]. In each PCR cycle, the temperature of reaction mixture is transferred between three zones (Figure 2.8):

Figure 2.8: A typical temperature profile of a PCR cycle [10]

1. Denaturation temperature, usually about 90°C to 96°C, is the temperature at which single-stranded DNAs are released as the templates in the next round of DNA synthesis.
2. Annealing temperature, usually about 40°C to 60°C, is the temperature at which primers attach to templates.
3. Extension temperature, usually about 70°C to 75°C, is the temperature at which DNA synthesis occurs.

Optimum temperatures or temperature ranges for each stage of the PCR reaction depend on factors such as particular DNA or RNA being replicated, number of cytosine-guanine hydrogen bonds vs. number of adenosine-thymine bonds, enzyme used, and length of primer. Precise maintenance of these optimum temperatures can effect reaction in numerous ways. Insufficient heating during denaturing is a common reason for a PCR reaction to fail. Similarly, overheating of the sample during denaturing can result in excessive denaturation of the polymerase or other enzymes being used [28].

During PCR cycle, annealing temperature is most important as it affects the specificity of reaction. DNA-DNA hybridization is a temperature dependent phenomenon. If the temperature is too high, no hybridization takes place; instead primers and templates remain dissociated, which results in decreased yield of the

desired product. However, if the temperature is too low, mismatched hybrids will occur, which are those not having all the correct base pairs formed. If these mismatches are tolerated, the number of potential hybridization sites for each primer is greatly increased, and the amplification is more likely to occur at non-target sites on template molecule, thus resulting in non-specific DNA fragments to be amplified. Ideal annealing temperature must be low enough to enable hybridization between primer and template but high enough to prevent mismatched hybrids from forming. This temperature can be estimated by determining melting temperature of primer-template hybrid [28, 29].

Attaining the optimum temperature for extension reaction is also important for obtaining desired PCR results. Temperature may affect both the rate and accuracy of extension reaction. If the rate of polymerase reaction is too slow, newly synthesized polynucleotide may not contain a site for primer annealing. Additionally, denatured polynucleotide sequence for amplification may contain one or more regions of secondary structure that may form or disappear according to temperature selected. Several different enzymes with polymerase activity may be used. Each enzyme will have its own optimum temperature for activity, stability and accuracy [10, 28].

Therefore, the determination of optimum temperatures is important to guarantee the success of PCR. In addition, any unnecessary temperature transition should be avoided. This inability to perform rapid transitions between temperatures causes inaccurate PCR results. A lag time between denaturation and annealing can result in unspecific annealing. Long transition times between denaturation and annealing also promote the premature inactivation of polymerase enzyme, resulting in less specific reaction and lower product yield [10]. So the rapid process of temperature transition should be improved, not only for saving time, but also for increasing the purity of PCR product. Temperature/time suggested for rapid cycle PCR are listed in Table 2.1.

Step	Temperature (°C)	Time (s)
Denaturation	94	0
Annealing	30 + 0.5(primer %GC)	0
Extension	74	0.03 (product length)

Table 2.1: Temperature/time suggested for rapid cycle PCR [30]

2.6 DNA Melting Analysis

A product melting curve can be obtained by continuously monitoring the fluorescence of each sample as it is heated at near-equilibrium rates of 0.05-0.2°C/s in a temperature range after PCR process (Figure 2.9) [30, 31].

Figure 2.9: PCR and DNA melting temperature profile obtained from Roche Molecular Lightcycler

As products melt, the fluorescence decreases (Figure 2.10 left). The temperature at which the rate of change of fluorescence ($-dI/dT$) is greatest is defined as the melting temperature (T_m) of the product [32, 33]. By taking differential curves (Figure 2.10 right), melting temperatures can be presented as peaks, which are analogous to the bands on an electrophoresis gel. [26]

Figure 2.10: Standard melting curves obtained from Roche Molecular Lightcycler

Figure 2.11: Melting curves with different GC/AT ratio. Amplicons of 24%, 34%, 44%, 54%, and 64% GC content, and 150bp in length, were amplified in the presence of 0.5X SYBR Green I dye.

Figure 2.12: Melting curves with different length. Graphing the negative first derivative ($-dI/dT$) of the fluorescence intensity for the 100bp, 500bp, and 1000bp amplicons calculates respective melting temperatures of 79.6°C, 83.8°C, and 86.6°C.

PCR products can be differentiated by analysis of melting curves which are functions of GC/AT ratio (Figure 2.11), length (Figure 2.12) and sequence. The melting temperature (T_m) of the amplicon defined by a primer pair is computed from the standard equation in Bolton and McCarthy as presented in Sambrook, Fritsch and Maniatis, Molecular Cloning [91,92]:

$$T_m = 81.5 + 16.6(\log_{10}([Na^+])) + 0.41(\%GC) - 600/\text{length}$$

where $[Na^+]$ is the molar sodium concentration, %GC is the percent of Gs and Cs in the sequence, and length is the length of the sequence. Empirical formulas [34] predict that a 0% GC duplex would melt 4°C lower than a 10% GC duplex. Given the same GC content, a 40-base-pair primer dimer should melt 12°C below a 1000-bp product [35].

Unlike gel electrophoresis, melting curve analysis can also distinguish products with different GC/AT sequences. Two products with the same length and GC content, but differing in GC distribution would have very different melting curves. Small sequence differences may be observable. For example, single-base differences in heterozygous DNA result in temperature shifts of 1°C–5°C for the lowest melting temperature domain of heteroduplexes compared to fully complementary DNA [35].

2.7 Temperature control of DNA melting analysis

Currently, PCR products with T_m 's differing by 2°C can be distinguished clearly within mixtures (Figure 2.13) [36]. Subsequent work suggests that even smaller

differences can be detected, including T_m shifts from heteroduplexes caused by single-base substitutions [37].

Figure 2.13: Melting curves and melting peaks for mixtures of amplification products. Melting curves (a) were acquired on a purified 180-base pair fragment from the hepatitis B surface antigen gene (A), a 536-base-pair fragment from the human b-globin gene (C), and a mixture of the two (B). The melting curves for the pure and mixed products were transformed into first-derivative melting peaks (b) [36].

The melting behaviour of purified PCR product is affected by the concentration of dsDNA dye and the rate of temperature transition during denaturation [32]. As the concentration of dye increased, the melting curves shifted to higher temperature. Rapid temperature transitions through the denaturation temperature also shifted melting curves to a higher apparent T_m because of kinetic effects (Figure 2.14).

Figure 2.14 Effect of temperature transition rate on melting curves. Melting curves were acquired at temperature transition rates of 0.1 (E), 0.2 (F), 0.5 (G), 1.0 (H), and 5.0° C/s (I). Each curve was normalized to range from a relative fluorescence of 2 at 84° C to 0 at 92° C [32].

Temperature transition rates of 5° C/s increased apparent T_m by nearly 3° C. The apparent T_m only shifted 0.2° C by doubling the transition rate from 0.1 to 0.2° C/s. [32] Slow temperature transition during DNA melting analysis results in accurate

detection of melting temperature. Rates of 12° C/min (0.2° C/s) can be used to differentiate products differing by 2° C or less in melting temperature [38], but for more precise DNA analysis, detection with higher temperature resolution and slow temperature transition should be carried out.

2.8 Review of commercial PCR and DNA melting analysis devices

Conventionally, standard PCR-based DNA assay is performed with a programmable thermal cycler, those heats and cools a tube, capillary or slide containing DNA sample mixture. The PCR amplification product is delivered to a detection device or location for fluorescence or gel electrophoresis measurement. Most of available PCR thermal cyclers are no longer large desktop devices. Today, there are many thermal cyclers available on market, all with features that are designed to make PCR faster and more accurate.

The Primus Thermal Cyclers from MWG Biotech, are equipped with most up-to-date High Temperature Range (HTR) Peltier technology. Phenomenal performance is rapid heating rates at 4° C/s and unique block homogeneity (+/-0.5° C). Rotor-Gene 3000 is a Real-time Amplification machine from Corbett Research Company [39], in which the rapidest PCR cycle is 1min. The temperature precision is +/- 0.1° C during DNA melting analysis, but the ramping control accuracy is 1° C. Roche Molecular Biochemicals Lightcycler is produced by Roche Company [40], in which the fastest ramping rate is 20° C/s. The temperature resolution is 0.1° C in DNA melting analysis. Roche LightTyper is also produced recently by Roche Company [41]. A proprietary heating block in the LightTyper slowly and consistently increases the temperature by as little as 0.05° C/sec, generating melting curves that are capable of discriminating even single-base changes by probe-melting temperature shifts of 2° C or greater. DNA Engine Opticon system from MJ Research Company is another machine for PCR and DNA melting curve analysis. Its ramping rate limit is 30° C/s, its temperature accuracy is +/-0.3° C, and the control precision is +/-0.4° C [42]. Bio-Rad iCycler is a real-time quantitative PCR machine from Bio-Rad Company. The temperature accuracy is

0.5°C [43]. ABI PRISM 7700 is produced by Applied Biosystems Company. Its temperature accuracy during DNA melting curve analysis is 0.5°C [44].

2.9 Disadvantages of conventional PCR devices

There are the following common restrictions associated with conventional PCR devices:

1. Slow thermal cycling during PCR process

The basis of most commercial PCR devices is intimate contact between a PCR reaction chamber holding sample and a heating block which is rapidly heated and cooled [28]. This heating/cooling block often has a large heat capacity, which limits the rate at which samples are heated and cooled [45, 46]. In addition, conventional systems require relatively high volume reaction (>10µL), which contributes to slower thermal cycling [47, 48]. In practice, overheating and sub-cooling cannot be entirely avoided. Furthermore, the greatest problem with PCR carried out on plastic PCR plates (e.g. 96 well-plates) is that the temperature gradients within the chamber plate may be as high as 10°C [6].

2. Low temperature resolution during DNA melting analysis

Most commercial products for PCR and DNA melting analysis use thermal couple to detect temperatures. First of all, the temperature resolution of thermal couple is low. Secondly, temperature sensor located outside PCR chamber detects the temperature of itself, which is not same with PCR sample. These causes temperature offset and time lag during DNA melting analysis.

3. High power consumption and large size

High power consumption and size of conventional PCR thermal cyclers are two other restrictions of preventing these devices from becoming portable for field use.

4. High risk of contamination

PCR products are delivered with pipettes into detection devices. These sample handling activities increase the risk of contamination of PCR products by surrounding environment, and subsequently affect the accuracy of DNA assays.

2.10 PCR microchips

Miniaturization concepts have recently been brought to the forefront of analytical sciences. Kalinina et al. had tested 10 nL of PCR volume and it was able to generate the sufficient concentration of PCR product after 30 cycles. This shift toward micro- and nano-scale experiments is driven partly by the increasing costs of samples and reagents, but mostly by the constantly improving sensitivity of modern analytical equipment [49]. In recent years, many devices for PCR have been constructed to demonstrate the benefits of miniaturization. These devices are commonly referred to as 'PCR microchips'. Based on micro-electro-mechanical-systems (MEMS) technology, the PCR microchips provide a high performance for PCR amplification and fluorescence detection [50, 51].

A number of micro-devices have been developed to accomplish faster PCR thermal cycling by reducing or eliminating large thermal mass present in macroscopic systems. One of the earlier devices developed is from Poser et al [52]. They developed chips with one, two and ten chambers with 5 μ L to 10 μ L volumes to investigate on the feasibility of miniaturizing thermal cyclers [52]. A unique feature of this design is inclusion of an air chamber, which insulates the reaction chamber to prevent heat loss by conduction to surrounding material, thus allowing a more uniform temperature distribution within the reaction chamber. From results obtained, Poser et al. concluded that miniaturized thermal cyclers had several advantages, namely rapid heating/cooling rates and consequently short cycle times. Miniaturized thermal cyclers with locally optimized heat flow are also found to be a very efficient tool for precise management of reaction conditions.

Another type of PCR microchip is a continuous flow PCR device developed by Kopp et al. [53]. This device has a channel on three different temperature zones, where temperatures are constantly maintained. Sample was processed across each temperature zone in turn. Thermal variation of heating blocks is unnecessary. This is an excellent approach as heating and cooling rates are directly affected only by heat capacity of sample solution, and not affected by heating/cooling step of chamber component and heating/cooling block. However, it is difficult to modify the processing time at each PCR step.

Associating the constant temperature control method for rapid PCR as used by Kopp et al. [53], Nagai et al. [45] fabricated a silicon based PCR chamber array by semiconductor technology. This device consists of numerous miniaturized PCR chambers, whereby each chamber is of pico-liter quantity. On average, the pico-liter chamber will contain on average one molecule, thus PCR amplification could be performed on the single molecule with only one template in mixtures containing various kinds of templates without competitive inhibition by other template [45]. Three hotplates, constantly controlled at 95°C, 55°C and 72°C, are utilized. Thus, large thermal mass that is commonly associated with heating/cooling block is eliminated. The device is repeatedly moved on hotplates. This heating and cooling process is found to be capable of attaining a maximum rate of approximate 16°C/s. Throughput could be enormous with the integration of more microchambers.

Similar to the constant temperature control method used by Kopp et al. [53], Chou et al [54] has designed and fabricate a miniaturized cyclic PCR device (Figure 2.15). The device comprises of a serpentine channel with different cross-sectional areas in different reactor zones to provide adequate residence time for denaturing (Reactor 1), annealing (Reactor 2) and extension (Reactor 3) to take place. Sample is manipulated using a peristaltic pump. The device could achieve 30 cycles in 40min with flow rate of 15µL/min.

Figure 2.15: Design of the cyclic PCR chip (top view) [54]

A micro-machined thermal reactor is presented by Zou et al [50]. It has a new thermal isolation design to eliminate thermal cross talk between reactor and substrate, and to reduce parasitic heat capacitance, thus the silicon substrate could remain unheated. The heat transfer is dominated by conduction in silicon beams, resulting in faster heating/cooling rate. The ramping rate is $14^{\circ}\text{C}/\text{s}$, and the cooling rate is $10^{\circ}\text{C}/\text{s}$.

Using an infrared-mediated temperature control, Giordano et al. [46] developed a polyimide microchip to carry out PCR. In his experiments, heating and cooling rates of $20^{\circ}\text{C}/\text{s}$ were achieved.

Maluf et al [47] mentioned that the high thermal conductivity of silicon would allow fast thermal cycling. Its capability to produce chambers of a high degree of accuracy and uniformity makes it desirable for PCR chip material [29]. Taylor et al. [56] have demonstrated a real-time, homogeneous, sequence specific detection of PCR products in silicon microstructures. A rapid thermal cycling was optimized in which temperature ramp rates of $4^{\circ}\text{C}/\text{s}$ was attained and cycle time was reduced. However, silicon poses a problem of material compatibility because enzyme binding to surface could cause some degradation in the overall quality of the chemistry. Nonetheless, many microfabricated devices with multi-analytical functions have been reported on glass, silicon-glass and silicon-plastic materials [4, 29, 48, 50, and 55].

Glass is another commonly used material in microfabrication of PCR chip. Taylor et al. [57] also optimized the PCR efficiency in glass microstructures with real time

detection to analyze device performances. Based on surface and bulk analytical techniques, chromium is identified as the primary contamination responsible for failures in PCR. This chromium is a by-product of the microfabrication processes. Therefore, Taylor et al. modified the original fabrication method to minimize chromium contamination. Changes included a decrease in the number of metal layers used in the metal mask, increased distilled water rinsing at all etch steps and a dilution chemistry technique for metal mask stripping step. As the result, the microstructure displayed efficient PCRs.

Lin et al. [58] demonstrated the use of finite element analysis to simulate the thermal characteristics of PCR chips with the aim to reduce thermal cycling time. Based on the results of the simulation, a chip was developed. Subsequently, an improved thermal profile with 5s denaturation and annealing holding time and zero holding time in primer extension was achieved.

Shinohara et al [6] has developed a micro chamber array for PCR. The concept of their development is to attain high throughput from 96 micro reaction chamber array (Figure 2.16). A finite element thermal analysis was performed to optimize the microchip design, so as to achieve precise temperature control of each chamber and each chamber array. As the result, heating rate of 10°C/s was attained.

Figure 2.16: Schematic (left) and cross sectional view (right) of the micro reactor array [6]

But during temperature transitions, the biggest temperature difference between the center node and boundary node of the sample is 5°C, because of the large thermal mass between center part of the sample and the Ti-heater. Another problem is the obvious thermal cross-talk, which results in imprecise temperature control.

Recently, Zou et al [59] develop a chip which is capable of independent thermal control in multiple reaction chambers, unheated substrate during thermal cycling, excellent temperature uniformity inside the reaction chamber and real-time on-chip monitoring of temperature distribution. The chamber volume is 0.1~20µL. The ramping rate is 14°C/s, while the cooling rate is 10°C/s. The temperature uniformity is $\pm 0.3^{\circ}\text{C}$.

In 2002, Quanbo Zou etc. [5] developed a 4×4 multichamber PCR microchip (Figure 2.17). A 30 cycles PCR can be finished in 15min, and the temperature accuracy is $\pm 0.1^{\circ}\text{C}$.

Figure 2.17: Structural design of the multi-chamber thermal cycler [5]

But the temperature sensor is placed below the balancing block and plastic substrate, where is blocked off from the reaction chamber by a large thermal mass. The temperature detected can not represent the real temperature of PCR sample. It causes the inaccurate temperature control of PCR process. In addition, the PCR products have to be analyzed by agarose gel electrophoresis, which results in potential contamination and subsequent inaccuracy of DNA assay.

2.11 Advantages of PCR miniaturization

As shown in the previous section with various PCR chip configurations and capacities, the PCR miniaturization can bring the following advantages:

1. More precise temperature control

Microsystems are more suited for precise temperature control than conventional systems due to its lower thermal mass and shorter thermal paths. Heater and sensors for local temperature measurement can be located closely or connected to chambers. This can provide a more accurate sensing on sample temperature. The choice of geometries and materials in microsystems allows configurations for well-controlled local heat flow during denaturing, annealing and extension processes [52, 60].

2. Faster thermal cycling

Since heating elements and cooling elements possess small volumes and small heat capacities, achieving high cooling and heating rates of 15°C/s - 40°C/s is possible [46, 52]. Ultimately, the minimum reaction time is only limited by the speed of enzyme reaction itself [61]. In addition, since sample volumes have shrunk significantly to the range of micro-litre or less, sample thermal cycling also becomes faster [46, 51].

3. Cost reduction

The cost of reagent is reduced due to microscale reaction volumes. System costs are also much lower due to the introduction of batch fabrication technologies. This allows single-use systems to be produced as well [8, 51].

4. High throughput

Coupled with the reduced analysis time, reduced reagent and fabrication costs, miniaturization can result in an increase of throughput. Appropriate microchip design could allow multiple samples to be tested.

5. Less waste

The coupling of traditional sample preparation with microchip electrophoresis is wasteful. For example, 20 μ L - 50 μ L of PCR product is generated with conventional thermal cycling. However, microchip analysis requires as little as 1 μ L of sample with only a few pico-liters actually consumed. In the case of PCR microchip, only a small volume of sample is needed for thermal cycling [46].

6. Ability to integrate with various platforms

Sample preparation, PCR thermal cycling, PCR product detection and post-processing can be integrated onto a micro-fluidic chip. This means that the risk of cross-contamination and infection due to sample handling will be reduced. Execution of the analysis will be faster through reduced manual manipulation [62].

Chapter 3 Methodology for PCR chip design and operation

3.1 Conceptual chip design and operation

As discussed in the previous chapter, a wide range of biologic and chemical analytical devices has been demonstrated to show the feasibility of molecular biological assays in micro-scale environments. Similar to these micro systems, the PCR microchip can be a monolithic system in which an array of PCR elements are fabricated onto a single substrate, performing rapid PCR processes and precise DNA melting analysis.

3.1.1 Design considerations for PCR microchip

The following design criteria are highlighted and will be taken into considerations during PCR microchip design.

3.1.1.1 Considerations in on-chip rapid PCR process

- **Thin film temperature control system**

Conventional PCR instruments, as mentioned before, often use a large heating/cooling block with only one thermocouple inside to control the temperatures of PCR samples. This causes obvious thermal delay. But in the PCR microchip, the micro-scaled thin film heater and sensor are located much closer to PCR samples which minimize the thermal mass of the PCR element; and thus temperature inside the element would be precisely controlled to perform higher heating and cooling rates.

- **Leakages**

Leakages on the chip could result in sample contamination and inhibition of PCR subsequently. Risk of contamination would be reduced greatly in a well-sealed PCR microchip using microfluidics technologies

- **Amplification of the desired DNA molecules**

Extreme sensitivity of PCR increases the probability of false priming, DNA contamination and wrong amplification. An approach to reduce this problem is to make the PCR processing in an extremely controlled environment and to purify the reagents and primers prior to use. However, the sample itself may contain components that interfere with PCR or cause false priming.

- Temperatures transition time

As mentioned in section 2.5, slow temperature transitions between the PCR stages cause inaccurate results during DNA melting process. Thus the temperature transition time should be minimized for micro scale reaction.

- Cycle time

Cycle time is generally defined as the time to accomplish a complete PCR cycle. It is a criterion how quickly the temperature of sample can be changed. So, the cycle time can be minimized in a micro PCR chip.

- Chip costs

Low cost is very important to produce disposable micro PCR chips. All materials and the corresponding fabrication processes employed in the chip production should be easily available and low cost.

- Throughput

The ability to perform PCRs in a PCR chamber array would greatly increase the throughput of genetic analysis. The simultaneous comparison with different types of DNAs is a significant contribution to PCR research.

- Thermal cross-talk between adjacent PCR elements

In a PCR array chip, the problem of the thermal cross-talk between adjacent PCR elements can not be ignored. It must be guaranteed that the PCR process in one

element is not influenced by those in adjacent elements. A direct method to reduce the thermal cross-talk is to use high thermal resistance materials between PCR elements.

3.1.1.2 Considerations in on-chip precise DNA melting analysis

- Precise temperature sensing and control

High resolution temperature sensor and controller are critical in a precise DNA melting analysis. In microchips, thermal mass between the sensor and PCR sample is in micro scale and this reduces the inaccurate results of DNA melting temperature greatly. Two DNAs differing by 2°C or less in melting temperature can be differentiated.

- Uniformity of sample temperature

Uniformity of sample temperature within a PCR chamber is very important for reliability and reproducibility of DNA melting analysis. Otherwise, different DNAs with different melting temperatures in the same chamber would melt at same time and can not be differentiated, since the temperature of one chamber is assumed uniform in the DNA melting analysis process.

3.1.2 Chip design specifications

Based on the design considerations, the initial specifications of PCR microchip are listed as the following:

1. The sample volume is set as 1 μ l.
2. The dimension of a single PCR element is 9mm $\times$ 9mm (the same dimension with one chamber in a standard plastic 96 well plate). Thus, it is portable, low cost and compatible.
3. Temperature ramping rate should be faster than 10°C/s, which is achieved by most developed PCR microchips.
4. Ability to perform at least 30 cycles of PCR amplification.

5. The temperature control accuracy should be less than 0.1°C during PCR thermal cycling process.
6. The temperature uniformity within each chamber during PCR thermal cycling process should be less than 10°C .
7. The temperature shift caused by thermal cross-talk between adjacent PCR elements should be less than 0.1% of the temperature difference between two PCR stages.
8. Precise temperature control up to 0.01°C during DNA melting analysis.
9. The temperature uniformity of the PCR sample during DNA melting analysis should be less than 0.01°C .

3.1.3 Conceptual chip design

Mentioned in section 3.1.1, the most important consideration for chip design is performing rapid PCR process and precise DNA melting analysis in PCR chamber array. Thus, based on the investigations on methods of heat transfer and chip materials, the conceptual design for PCR microchip is illustrated as Figure 3.1. The material for the top layer, the bottom layer and the substrate is glass, and the material for the cover, wall and boundary is the SU-8 photoresist.

A unique feature of this design is that the top layer, bottom layer and substrate are made of extremely thin plates with thickness in the order of micrometers. In this configuration, the thermal mass in PCR elements is reduced greatly. Thus, the heat conduction from heater to PCR sample will be much faster.

The second feature of this design is the convection channel in every PCR element, which has not appeared in any previous PCR microchips. With the forced air convection for each PCR element, cooling would be more efficient, at the same time the cooling rate and duration can be individually controlled.

Figure 3.1: Conceptual designs of PCR microchip (a) Sketch of micro PCR system; (b) 3D model of PCR multichamber array chip (96 PCR elements); (c) Cutaway view of a single PCR element from A-A interface (as shown in Figure (d)); (d) Cross-sectional view of single PCR element and airflow for cooling; (e) Temperature profile for PCR thermal cycling; (f) Temperature profile for DNA melting analysis.

The third feature is that the inner diameter of the element is much larger than its thickness, so the chamber is extremely thin. A small heat input from the bottom

surface of PCR sample can be transported to the top surface of PCR sample immediately, and thus the temperature uniformity of the PCR sample is excellent and a precise temperature control can be achieved easily.

The fourth novel feature is that the structure of a single PCR element can be duplicated to form an array of multiple PCR elements. Every element is an integrated and independent PCR system with minimum thermal cross-talk.

3.1.4 Chip material

Micro devices can be constructed by a multitude of materials depending on their unique characteristics and properties. Since a CCD camera should catch images above the microchip to detect the fluorescence intensity in PCR process and DNA melting analysis, materials of proposed microchip must be transparent and not illuminant. To achieve rapid heating and cooling, a material with high thermal conductivity and convection coefficient is selected for substrate construction. Another material with high thermal resistance is needed for wall and boundary construction to reduce the thermal cross-talk between the PCR elements.

3.1.4.1 Glass

Glass is selected to construct substrate of the chip due to its suitable thermal conductivity, biochemical compatibility with PCR chemistry, and its ability to be microfabricated using standard wafer fabrication process. [64] Table 3.1 shows its material properties useful to this work.

Table 3.1: Properties of glass (96 % silica glass) [51]

Properties	Data
Density	2180 kg/m ³
Heat capacity	0.75 kJ/kg·K
Thermal conductivity	1.1 W/m·K
Melting point	1020°C
Acid reaction	hydrofluoric acid

3.1.4.2 SU-8 photoresist

SU-8 is a high contrast, epoxy based photoresist for micromachining and other microelectronic applications, where a thick chemically and thermally stable image is desired. The exposed and subsequently cross-linked portions of the film are rendered insoluble to liquid developers [65]. Because of its desirable properties (Table 3.2) of thermal/electrical insulation, SU-8 photoresist is selected in this work to reduce thermal cross-talk between adjacent PCR elements.

Table 3.2: Properties of SU-8 [51]

Properties	Data
Density	1200 kg/m ³
Heat capacity	1.5 kJ/kg·K
Thermal conductivity	0.2 W/m·K

3.1.5 Methods of heat transfer

There are three broad categories of heat transfer mechanisms that can be used for thermal cycling of a PCR chip, namely conduction, convection and radiation (Appendix C). Comparing the three methods of heat transfer, direct conduction is selected for heating in the proposed microchip because of its rapid temperature ramping, precise temperature control ability and high heat transfer efficiency, as well as easy operation. A platinum microheater [63] is available from Heraeus Sensor Company for use in this work, in addition to our own microfabricated thin film heater. The microheater has a rapid temperature ramping rate (200°C increase in 300ms with the current less than 2A), excellent long term stability (max. R₀-drift is 0.04% after 1000h at 500°C), wide operating temperature range (-40°C to 500°C) and small tolerance (+/-0.5%). In this project, a similar Platinum thin film microheater deposited on the surface of substrate was fabricated as the heat source of the proposed microchip.

For efficient cooling, forced convection is selected. An air stream at room temperature can be directed into each PCR element to enhance heat loss.

3.1.6 Principle and specifications of thin film RTD temperature sensor

The selection of temperature sensors generally focuses on accuracy, response time, stability and repeatability. According to the analysis in Appendix E, RTD temperature sensor is the most suitable for this chip design.

RTD temperature sensors have a very low thermal mass, thereby providing true surface temperature measurements as well as fast response time (50 milliseconds). The established position of platinum sensor as a precise temperature sensor is demonstrated by its selection to define the International Temperature Scale (ITS-90). The platinum can be deposited in a resistance pattern on a small area, even directly on the measurement surface [70]. Thus, a thin film RTD temperature sensor deposited on the surface of substrate is selected for the precise temperature detection in the proposed microchip.

3.1.6.1 Principle of RTD temperature sensor operation

The resistance of platinum RTD temperature sensor follows a linear relationship with temperature. RTDs operate on the inherent property of metals to exhibit a change in electrical resistance as a result of a change in temperature. This resistance, R , is directly proportional to a metal wire's length L and resistivity of material ρ , and inversely proportional to the cross sectional area, A , that is

$$R = (\rho L) / A \quad (3.1.1)$$

$$\text{Working principle of the RTD is given as } \rho_T = \rho_0 [1 + \alpha(T - T_0)] \quad (3.1.2)$$

where, ρ_T is the resistivity at temperature T ;

ρ_0 is the resistivity at temperature T_0 ;

α is the temperature coefficient of resistance ($^{\circ}\text{C}^{-1}$).

Setting $T_0 = 0^{\circ}\text{C}$, we have

$$\frac{R}{R_0} = \alpha T + 1 \quad (3.1.3)$$

where R_0 is the resistance at temperature T_0 , α is a constant ramp.

The temperature coefficient is often defined as the mean temperature coefficient between 0°C and 100°C which represents the average change in resistance [70].

Therefore, from equation (3.1.3),

$$\alpha = \frac{R_{100} - R_0}{R_0 \times 100^\circ\text{C}} \quad (3.1.4)$$

The Platinum RTDs are made of IEC/DIN-grade platinum, and the ramp $\alpha = 0.00385\Omega/\Omega/^\circ\text{C}$ [71].

3.1.6.2 Specifications of thin film RTD

When discussing RTDs, several specifications must be considered:

- Self-heating

To measure electrical resistance of a RTD, it is necessary to pass an electrical, I . The resultant voltage drop across resistor heats the device in an effect known as I^2R or Joule heating. But self-heating offset between indicated temperature and actual temperature is too small and often neglectable.

- Accuracy

IEC standard 751 sets two tolerance classes for the interchangeability of platinum RTDs: Class A and Class B:

Class A: $\Delta t \text{ }^\circ\text{C} = \pm (0.15 + 0.002 \cdot |t|)$

Class B: $\Delta t \text{ }^\circ\text{C} = \pm (0.30 + 0.005 \cdot |t|)$

where, $|t|$ = absolute value of temperature in $^\circ\text{C}$

Class A applies to temperatures from -200°C to 650°C , while Class B covers the entire range from -200°C to 850°C .

- Resolution

Measurement resolution of 0.001°C is achievable after calibration by PT-100 sensor using PT-104 Platinum resistance data logger (Pico Technology Limited, UK) (please refer to Appendix G and reference [69] for detailed information). With detection of 0.1 ohm among 1000 ohm resistance, resolution of 0.01% would be achieved.

- Stability

Stability is the sensor's ability to maintain a consistent output when a constant input is

applied. Physical or thermal shocks can cause small, one-time shifts. Stability limits conservatively specified are typically $0.05^{\circ}\text{C}/\text{yr}$ in wide temperature ranges or $0.01^{\circ}\text{C}/\text{yr}$ in medium ranges.

- Repeatability

Repeatability is the sensor's ability to give the same output or reading under repeated identical conditions. In platinum RTDs, cycle to cycle differences normally can't be measured and are considered lumped into stability specifications.

- Response time

Response time is the sensor's ability to react to a change in temperature, and depends on the sensor's thermal mass and heat transfer from the material being tested. Thin film RTDs deposited on a surface respond quickly to surface temperature change and the time can be ignored.

3.2 On-chip thermal cycling process

3.2.1 Analysis of PCR thermal cycling process

A PCR thermal cycle (Figure 3.2) can be described as 6 stages listed as Table 3.3.

Figure 3.2: A typical temperature profile of a PCR and the different stages of a PCR thermal cycle.

Table 3.3: Stages of a PCR cycle

Stage 1	Temperature transition from Denaturation to Annealing	Thermal transition process
Stage 2	Denaturation stage	Steady-state heat transfer process
Stage 3	Temperature transition from Annealing to Extension	Thermal transition process
Stage 4	Annealing stage	Steady-state heat transfer process
Stage 5	Temperature transition from Extension to Denaturation	Thermal transition process
Stage 6	Extension stage	Steady-state heat transfer process

3.2.1.1 Steady-state heat transfer stages (2, 4, 6)

According to Appendix C, the thermal radiation from PCR microchip to the surrounding is almost neglectable. Only the heat conduction and the convection (Table 3.4) are considered in models using in this work.

Table 3.4: Heat transfer modes during steady-state heat transfer processes

STAGE	HEAT TRANSFER BOUNDARY	HEAT TRANSFER MODES
Denature (stage 2) Annealing (stage 4) Extension (stage 6)	Top surface, bottom surface, convection channel	Forced air convection
	insulation wall	Heat conduction
	Heater area	Heat source

To simplify thermal networks of the complex model, composite walls (Figure 3.3) are investigated firstly. A series of $n-1$ slabs of dissimilar materials in intimate contact are located between two constant temperature $T_{1,e}$ and $T_{n,e}$. Assuming no heat sources or sinks, Equation (3.2.1) applies to all layers,

$$-q_x = k \frac{dT}{dx} \quad (3.2.1)$$

Thus heat flux q_x is a constant at any cross section (any position x) in Figure 3.3.

Moving from left to right, there exists in each subregion an equivalent expression.

$$\begin{aligned} q_x &= (k_{1,2})(T_1 - T_2)/(x_2 - x_1) = (k_{2,3})(T_2 - T_3)/(x_3 - x_2) \\ \dots &= (k_m)(\Delta T_m)/(\Delta x_m) \dots = (k_{n-1,n})(T_{n-1} - T_n)/(x_n - x_{n-1}) \end{aligned} \quad (3.2.2)$$

where k_m is thermal conductivity of material for layer m . According to Equation (3.2.2), if the layer thickness Δx_m ($\Delta x_m = (x_m - x_{m-1})$, m is from 2 to n) decreases, the temperature difference between two surfaces of the layer ΔT_m ($\Delta T_m = (T_{m-1} - T_m)$, m is from 2 to n) decreases proportionally. Thus, a small layer thickness causes better temperature uniformity in chip.

Figure 3.3: Schematic of composite walls problem (a) Sketch of PCR microchip; (b) Model of composite walls [91].

Additionally, $\frac{(\Delta x_m)}{(k_m)A}$ is defined as the thermal resistance [74], where A is the layer area. Because k_m is a constant determined by material, $(\Delta x_m)/A$ should be minimized for less thermal resistance in microchip. Thus the horizontal dimensions of microchip must be designed much bigger than the vertical dimensions.

3.2.1.2 Thermal transition stages (1, 3, 5)

According to the conservation of energy, $\dot{E}_{in} + \dot{E}_g - \dot{E}_{out} = \dot{E}_{st}$ (3.2.3)

where $\dot{E}_{in}$ and $\dot{E}_{out}$ are the rates at which thermal energy enter and leave a surface, $\dot{E}_g$ is the rate of energy generation and $\dot{E}_{st}$ is the rate of change of energy stored.

Assuming $\dot{E}_{in} = 0$ and $\Delta \dot{E} = \dot{E}_{st}$, $\Delta \dot{E} = \dot{E}_g - \dot{E}_{out}$

Since a change in energy storage is due to temperature change,

$$\dot{E}_{st} = \rho Vc \frac{dT}{dt} \Rightarrow \Delta \dot{E} = \sum \rho Vc \frac{dT}{dt}$$

where ρ is the density of the object (including the PCR sample, SU-8 and glass structures), V is the volume of the object, c is the specific heat capacity of the object, T is object average temperature and t is time.

According to Equation (2) in Appendix C,

$$\sum \dot{E}_{out} = \sum hA(T_w - T_\infty) \text{ and } \dot{E}_g = P_{temp}, \text{ so Equation (3.2.3) can be expressed as}$$

$$\sum \rho Vc \frac{dT}{dt} = P_{temp} - \sum hA(T_w - T_\infty) \quad (3.2.4)$$

where P_{temp} is the rate of energy generation form heater, h is the convection heat-transfer coefficient, T_w is the object surface temperature and T_∞ is the fluid free-stream temperature, A is the object's exposed area.

During heating, according to Equation (3.2.4), the rate of change of energy stored into the microchip, $\Delta \dot{E} = P_{temp} - \sum hA(T_w - T_\infty) > 0$. To achieve faster heating rate dT / dt , ρVc , where ρ is the density of the object (including the PCR sample, SU-8 and glass structure), V is the volume of the object, c is the specific heat capacity of the object, should be minimized. Density ρ and thermal capacity c are determined by materials used, thus volume of PCR chip V is the most direct factor affecting heating rate. So the PCR chip should be microfabricated.

During cooling, according to Equation (3.2.4), the rate of energy generation, $P_{temp} = 0$, so the equation for conservation of energy is $\sum \rho Vc \frac{dT}{dt} = -\sum hA(T_w - T_\infty)$. Equation (3.2.4) can be rearranged as $\sum \rho c \frac{dT}{dt} = -\sum h \frac{1}{\lambda} (T_w - T_\infty)$, where the thickness of

object (including the PCR sample, SU-8 and glass structure) $\lambda = \frac{V}{A}$. So to achieve a

rapid cooling rate, thickness of PCR chip should be minimized.

As a summary, it is necessary to design a PCR chip to be fabricated in micro scale with large ratio of horizontal dimension to vertical dimension.

3.2.2 Investigation on thermal cross-talk in PCR chamber array

The thermal cross-talk between PCR elements should be minimized by a proper design of the insulation thermal paths formed by the substrate and the cover attached on to the PCR sample. Figure 3.4 (b) shows the thermal equivalent circuit, where R_{si} , R_{ci} and R_{cx} represent the thermal resistance between the i th heater and bottom layer, between the i th heater to the i th PCR sample, between two adjacent PCR elements, respectively.

Figure 3.4: Sketch (a) and equivalent circuit (b) of thermal cross-talk model [5], where R_{si} is the thermal resistance between the i th heater and bottom layer, R_{ci} is the thermal resistance between the i th heater to the i th sample, R_{cx} is the thermal resistance between two adjacent PCR elements, CM_i is the temperature of the i th sample, P_i is the power of the i th heater ($i=1,2$).

Assuming $R_{s1}=R_{s2}=R_s$, $R_{c1}=R_{c2}=R_c$ and $R_{cx} \gg R_{ci}$, R_{si} (which can be easily achieved with thin substrate and cover), the temperature in reaction element 1 and 2 (T_{CM1} and T_{CM2}) are

$$T_{CM1} = \frac{R_s R_{cx}}{R_{total}} [P_1 + P_2 (\frac{R_c + R_s}{R_{cx}})] \quad \text{and} \quad T_{CM2} = \frac{R_s R_{cx}}{R_{total}} [P_2 + P_1 (\frac{R_c + R_s}{R_{cx}})] \quad (3.2.7) [5]$$

Where $R_{total} = R_s + R_c + R_{cx}$. As $\frac{R_s + R_c}{R_{cx}} \ll 1$, the i th heating power P_i ($i=1,2$) will mainly heat up the i th sample (CM_i), with very small cross-talk to the other PCR elements.

Thus, to minimize the thermal cross-talk between PCR chamber arrays, the thermal resistance between adjacent PCR elements (R_{cx}) should be designed to reach the

maximum. Because thermal conductivity is depend on material, the most efficient method to increase thermal resistance is to increase the thermal path between PCR elements. The detailed temperature variation caused by thermal cross-talk in proposed chip is presented in section 3.4.2.

3.2.3 Thermal analysis of DNA melting process

After a PCR process, the sample temperature should be controlled precisely for DNA melting analysis. Because the reaction chamber in the chip is shallow, the effect of the natural convection of the sample inside a chamber on the temperature distribution can be ignored during PCR process, but not in the DNA melting analysis, since an extremely precision temperature control (up to 0.01°C) is to be achieved with an proposed chip. An investigation must be done before hand. The sample convection model is illustrated as Figure 3.5.

Figure 3.5: Natural convection model of sample, where L is the diameter of sample cylinder, l is the thickness of sample cylinder.

According to Appendix C, $Gr = \frac{g\beta\Delta TL^3}{\nu^2} = \frac{g\Delta TL^3}{Tv^2}$; $Nu = 0.60(Gr Pr)^{\frac{1}{5}}$; $h = Nu \frac{K}{L}$

Convection ratio is defined as,

$$\begin{aligned}
 r_c &= p / P = Ah\Delta T / P = \pi Ll h \Delta T / P = \pi l \Delta T Nu K / P = 0.60 \pi l \Delta T K (Gr Pr)^{\frac{1}{5}} / P \\
 &= 0.60 \pi l \Delta T K (Gr Pr)^{\frac{1}{5}} / P = 0.60 \pi l \Delta T K \left(\frac{g\beta\Delta TL^3}{\nu^2} Pr \right)^{\frac{1}{5}} / P \\
 &= 0.6 \pi K Pr^{\frac{1}{5}} g^{\frac{1}{5}} \beta^{\frac{1}{5}} \Delta T^{\frac{6}{5}} (L^{\frac{3}{5}} l) \left(\frac{1}{P} \right) \left(\frac{1}{\nu} \right)^{\frac{2}{5}} \quad (3.2.5)
 \end{aligned}$$

where Gr is Grashof Number, Pr is Prandtl Number, Nu is Rayleigh Number [74], g is acceleration of gravity, A is the area of sample curved side, L is the diameter of

sample, l is the thickness of sample, ν is kinematic viscosity, β is expansion coefficient, K is thermal conductivity, T is sample temperature, ΔT is the difference between the initial temperature and the final temperature, p is the power of convection, P is the power supplied by heater. From Equation (3.2.5), it is concluded that a smaller diameter and thickness of sample result in a lower convection ratio. The relationship between the convection ratio and parameters of sample is illustrated in Figure 3.6.

Figure 3.6: Convection ratio vs. diameter and thickness of sample

As shown in Figure 3.6, even if the diameter is 2cm and the thickness is 1mm, convection ratio is less than 0.045%. Thus, sample convection would be ignored during DNA melting analysis.

A heat conduction model of composite layers is used to analyze sample condition during melting (Figure 3.7). According to Appendix D, the temperature difference between the top node and the bottom node of the sample becomes,

$$\Delta T = (T_1 - T_0) \left(e^{-\left(\frac{\pi b}{2L_2}\right)^2 t} \right) \left(1 - e^{-\left(\frac{\pi^2}{4L_2^2}\right) \left[\frac{L_1^2 b^4 + 2L_1 L_2 a b^3}{b^2 L_1^2 + L_2^2 a^2 + 2L_1 L_2 a b^3} \right] t} \right) \quad (3.2.6)$$

where T_0 is initial temperature of the heater, T_1 is final temperature of the heater, L_1 is the thickness of the sample, L_2 is the thickness of the glass substrate, t is time.

Figure 3.7: Thermal diffusion model of two composite walls, where L_1 is the thickness of sample, L_2 is the thickness of glass substrate, a^2 is the thermal diffusivity of sample, b^2 is the thermal diffusivity of glass substrate, and arrows are the direction of heat transfer. SU-8 cover is assumed as a thermal insulation boundary. At the initial time, the temperature of heater increases from T_0 to T_1 .

For the sample, the thermal diffusivity can be expressed as $a^2 = \frac{K_1}{\sigma_1 \rho_1}$, where K_1 is the thermal conductivity, σ_1 is the specific heat, and ρ_1 is the density of material.

For the glass substrate, the thermal diffusivity can be expressed as $b^2 = \frac{K_2}{\sigma_2 \rho_2}$, where K_2 is the thermal conductivity, σ_2 is the specific heat, and ρ_2 is the density of material.

From Equation (3.2.6), it can be concluded that, to minimize the temperature difference between the top node and the bottom node of the sample (ΔT), t and a^2 and L_2 should be maximized, and L_1 and b^2 should be minimized.

The temperature difference between top and bottom nodes of the sample must be less than 0.001°C within 1 second, which is a criterion of the precise temperature control during the DNA melting analysis. The relationship between the temperature difference and the thicknesses of sample and glass substrate is illustrated in Figure 3.8.

Figure 3.8: Temperature difference between top and bottom of sample vs. thicknesses of sample and glass substrate at 1 second

Figure 3.9: Contour map of temperature difference between top and bottom of sample vs. thicknesses of sample and glass substrate at 1 second

As shown in Figure 3.8, the blue layer at 0.001°C level is a criterion level, and the surface above it can not be used in chip design. The part below the blue layer can achieve a good temperature uniformity and precise temperature control. Figure 3.9 gives a clear graph of these two parts. The yellow area oversteps 0.001°C at the end of

1 second, which is not suitable for chip design. Additionally, from Appendix D, the temperatures of the top and the bottom nodes of the sample are

$$T_{top}(t) = T_1 + (T_0 - T_1)e^{-\left(\frac{\pi a}{2(L_1 + \frac{b}{a}L_2)}\right)^2 t} \quad \text{and} \quad T_{bottom}(t) = T_1 + (T_0 - T_1)e^{-\left(\frac{\pi a}{2L_2 \frac{a}{b}}\right)^2 t}, \quad \text{which show that}$$

thicker layers (sample or glass substrate) cause longer thermal delay. Balancing with melting transition time, the thickness of layers should be limited in a range. So, the recommended range of the layer thicknesses for chip design is described as the blue area in Figure 3.8. The detailed thickness ranges of sample and glass substrate in proposed microchip are presented in section 3.4.2.

3.3 Development of proposed PCR chip

Based on the design considerations described in previous sections, the microchip concept is further developed and described in the following sections.

3.3.1 Single PCR element design

The single PCR element consists of three main layers with accessory structures illustrated in Figure 3.11 and 3.12. The single PCR element with the airflow for the forced convection cooling is illustrated in Figure 3.13.

3.3.1.1 Top layer with SU-8 cover

The top layer is a glass slide with SU-8 structure aiming to seal and protect the sample from contaminations. The diameter of the SU-8 cover is 4mm and the thickness is 120 μ m, while the dimension of glass top layer is 9mm $\times$ 9mm $\times$ 0.1mm.

3.3.1.2 Middle layer with SU-8 wall and boundary

The middle layer includes a PCR reaction chamber (composed by the SU-8 wall and the glass substrate) containing PCR sample, an air convection channel which is purposed to achieve faster cooling in PCR process, and a SU-8 boundary to eliminate the thermal cross-talk. The thickness of glass substrate is 0.1mm, while the thickness of the wall and the boundary is 0.2mm. Thus, as shown in Figure 3.10, the thickness of the PCR chamber should be 80 μ m, which is the difference of the wall thickness

and cover thickness. The inner diameter of the wall is 4mm, while its outer diameter is 5mm. The length of the boundary is 9mm, while its width is 0.5mm. Thus, the dimension of the whole middle layer is 9mm×9mm×0.3mm.

Figure 3.10: Cross-sectional view of a single PCR element

3.3.1.3 Bottom layer with microheater and RTD sensor

A Pt film RTD sensor and a Pt film micro-heater are deposited on the surface of the glass bottom layer serving as the temperature-sensing element and the heat source for the PCR and DNA melting processes. The heater and sensing area is within a disk of 4mm in diameter. The inner diameter of convection channel is 1mm. The dimension of the glass bottom layer is 9mm×9mm×0.1mm.

Figure 3.11: Top view of single PCR element

Figure 3.12: Bottom view of single PCR element

Figure 3.13: Sketch of assembled single PCR element with the airflow for forced convection cooling

3.3.2 Thermal characteristics of PCR process

It is assumed that the single PCR element contains only nine main thermal components: the microheater, the PCR sample, the SU-8 wall, the SU-8 cover, the glass substrate, the convection channel, the boundary, glass top layer and glass bottom layer. Every element is regarded as a thermal insulation system which is not influenced by ambience, and the heat loss via the boundary can be ignored. In the steady-state heat transfer processes, all energy from heater is taken away by forced air convection. By calculating convection heat loss, the power supplied by heat source can be determined.

According to Appendix C, the average convection coefficient is $\bar{h} = \frac{\overline{Nu_L} \cdot k}{L_c}$, where $\overline{Nu_L}$ is the average Nusselt Number, k is the thermal conductivity of medium, and L_c is the characteristic length. Different convection coefficients in PCR processes are listed as Table 3.5 and 3.6. Detailed calculations for these results are shown in Appendix A.

Table 3.5: Summary of the convective heat transfer coefficients during steady-state heat transfer stages

Steady-state heat transfer stage	2 $\bar{h} \left(\frac{W}{m^2 \cdot K} \right)$	4 $\bar{h} \left(\frac{W}{m^2 \cdot K} \right)$	6 $\bar{h} \left(\frac{W}{m^2 \cdot K} \right)$
Top and bottom surface	88.08	94.18	88.38
Convection channel surface	80.30	103.30	96.94

Table 3.6: Summary of the convective heat transfer coefficients during thermal transition stages

Thermal transition stage	1 $\bar{h} \left(\frac{W}{m^2 \cdot K} \right)$	3 $\bar{h} \left(\frac{W}{m^2 \cdot K} \right)$	5 $\bar{h} \left(\frac{W}{m^2 \cdot K} \right)$
Top and bottom surface	6.450	88.35	5.711
Convection channel surface	6.450	96.90	5.711

As shown in Table 3.5 and 3.6, stage 4 (annealing stage) exhibits the highest convection coefficient during a PCR cycle. The energy supplied by heat source should be more than these taken by cooling air convection to hold the temperature required in the annealing stage. According to Appendix C, Newton's Law of Cooling is expressed as

$$\dot{Q} = hA(T_w - T_\infty)$$

The heat loss by convection on top and bottom surfaces of microchip is given by

$$\dot{Q}_{outside} = h_{outside}A_{outside}(T_w - T_\infty) = 0.11332W \quad \text{and the heat loss in the convection channel}$$

$$\text{(Figure 3.12) is given by } \dot{Q}_{inside} = h_{inside}A_{inside}(T_w - T_\infty) = 0.09388W ; \text{ thus the sum of heat}$$

$$\text{loss is given by } \sum \dot{Q} = \dot{Q}_{outside} + \dot{Q}_{inside} = 0.20720W . \text{ Thus, when the air velocity is 4m/s}$$

(Papst fan 322-4423, EBMPAPST, Germany), the energy supplied by the heater should be more than 0.2072W. In this work, the power of heat source is set at 0.25W.

3.3.3 Microheater

The microheater operates under a constant-voltage and its resistance is given by

$$R = U^2 / P \quad (3.3.1)$$

where R is the resistance (Ω), U is the voltage (V), and P is the power (W). With a 0.25W power supply at 12V, the resistance of the micro heater equals to 576 Ω (at the room temperature, 20°C).

According to resistance correlation;

$$\frac{R}{\sigma} = \frac{L}{Wt} \quad (3.3.2)$$

where σ is resistance coefficient (Ωm), (for Pt film, σ is $9.6 \times 10^{-8} \Omega\text{m}$; for Al film, σ is $3.2 \times 10^{-8} \Omega\text{m}$). L is the length of the heater, t is the thickness, and W is the width (set as $100\mu\text{m}$). Therefore,

$$\frac{R}{\sigma} = \frac{L}{Wt} = \frac{576}{9.6 \times 10^{-8}} = \frac{L}{10^{-4}t}, \text{ where } L=24\text{mm, the thickness is } 400 \text{ \AA} \text{ and } \frac{L}{t} = 6 \times 10^5$$

The detailed pattern of the microheater is illustrated in Figure 3.14 and 3.15.

3.3.4 RTD temperature sensor

As mentioned in section 3.1.6, the resistance of Pt thin film RTD temperature sensors has a linear relationship with temperature, as given by

$$R=R_0 [1+ \alpha(T-T_0)] \quad (3.3.3)$$

where R is the resistance (Ω) at temperature T ($^{\circ}\text{C}$), R_0 is the reference resistance (Ω) at temperature T_0 ($^{\circ}\text{C}$) (the selection of the R_0 and T_0 can be found in Section 5.7), and α is the temperature coefficient of resistance (TCR), which is $0.0038/^{\circ}\text{C}$ [72]. To measure the temperature of the whole PCR sample, the Platinum sensor should be deposited as a serpentine line covering all areas of the sample bottom surface. The width of the sensor should be narrow enough to fit into spacing of heater pattern. Additionally, the thickness of the sensor must be the same as that of the heater because of the characteristics of the microfabrication technology. According to the resistance correlation (3.3.2), $\frac{R}{\sigma} = \frac{L}{Wt} = \frac{R}{9.6 \times 10^{-8}} = \frac{20}{0.05 \times 400 \times 10^{-10}} = 10^{10}$, with the width set at $50\mu\text{m}$ and the length set at 20.00mm , the resistance of RTD temperature sensor is 960Ω (at the room temperature, 20°C , the temperature sensor calibration is shown in Section 5.7). The specifications of the microheater and the RTD temperature sensor are listed in Table 3.7. The pattern of the RTD temperature sensor is illustrated as Figure 3.14 and 3.15.

Table 3.7: Specifications of microheater and RTD temperature sensor

Specifications	Micro heater	RTD temperature sensor
Thickness ($\text{\AA}$)	400	400
Width (μm)	100	50
Length (mm)	24.00	20.00
Resistance (Ω) (@20°C)	576	960

3.3.5 Leads

Copper is selected as leads of the heater and the temperature sensor. The thickness of the leads is $1\text{ }\mu\text{m}$, while the width is $200\mu\text{m}$. Because the resistance of the leads is less than $1/100$ of the micro heater, the self heating of the leads is neglectable. The pattern of the leads is illustrated in Figure 3.14 and 3.15.

Figure 3.14: Sketch of assembled single PCR element

Figure 3.15: Section view of assembly single PCR element

3.3.6 Assembled single PCR element

Based on the previous investigations, an assembled single PCR element, including the microheater, the RTD temperature sensor and the leads, is illustrated in Figure 3.14 and 3.15.

3.3.7 PCR chamber array

PCR chamber array as shown in Figure 3.1(b) can be made on a single microchip by assembling more than one single PCR elements. Thus, 96 PCR reactions under different PCR conditions can be conducted simultaneously for various molecular biology applications.

3.4 Thermal characteristics of proposed PCR microchip

3.4.1 Analytical investigation of thermal cycling during PCR process

Based on the investigations in section 3.2.1, the Equation (3.2.4) can be expressed as:

$$\rho_{sample} V_{sample} C_{sample} \frac{T_{final} - T_{initial}}{t} + \rho_{glass} V_{glass} C_{glass} \frac{T_{final} - T_{initial}}{2 * t} + \rho_{SU-8} V_{SU-8(in)} C_{SU-8} \frac{T_{final} - T_{initial}}{t} \\ = P_{temp} - [h_{outside} A_{outside} (T_w - T_{\infty}) / 2 + h_{inside} A_{inside} (T_w - T_{\infty}) / 2]$$

According to the chip specifications, the detailed parameters used in these equations are listed in the following:

Denaturation temperature is 95°C;

Annealing temperature is 55°C;

Extension temperature is 70°C;

Sample: $C=4.2\text{KJ}/(\text{Kg}\cdot\text{K})$; $\rho=1000\text{Kg}/\text{m}^3$; $V=1\mu\text{l}=1\times 10^{-9}\text{ m}^3$

SU-8: $C=1.5\text{KJ}/(\text{Kg}\cdot\text{K})$; $\rho=1200\text{Kg}/\text{m}^3$;

$V=2.669\times 10^{-6}\text{ m}^3$ during cooling process,

V is assumed to be zero during heating process, because the heating time is too short to transfer heat into SU-8 structure;

Glass: $C=0.7\text{KJ}/(\text{Kg}\cdot\text{K})$; $\rho=2200\text{Kg}/\text{m}^3$;

$V=24.064 \times 10^{-9} \text{ m}^3$ during cooling process,

V is assumed to be $3.925 \times 10^{-9} \text{ m}^3$ during heating process, because the heating time is too short to transfer heat from the center part to the boundary part of the glass layers;

Convection area of top surface and bottom surface: $A=126.43 \times 10^{-6} \text{ m}^2$

Convection area of air channel surface: $A=90.634 \times 10^{-6} \text{ m}^2$

Using Matlab (Version 6.0) programming, the graphs of temperature vs. time are illustrated in Figure 3.16 to 3.18. The duration from denaturation to annealing (stage 1) is 1.0244 seconds; the duration from annealing to extension (stage 3) is 3.5012 seconds; the duration from extension to denaturation (stage 5) is 0.6147 seconds.

Figure 3.16: Temperature transition during stage 1

Figure 3.17: Temperature transition during stage 3

Figure 3.18: Temperature transition during stage 5

Fans (Papst fan 322-4423, EBMPAPST, Germany) are commonly capable of achieving variable air velocities. Effect of air velocity on temperature transition during stage 3 is illustrated in Figure 3.19, which shows that the higher air velocities improve cooling rate significantly. But the variable incoming air velocity has no influence on the temperature transition during process 1 and 5 because the fan is off.

Figure 3.19: Temperature transition during stage 3 vs. incoming air velocity

The heater power supply outputs a variable voltage. The effect of the power supplied on temperature transitions is illustrated in Figure 3.20 and 3.21. A higher power supplied offers a higher heating rate. Because the heater is turned off during the cooling process, the variable power has no effect on temperature transition in stage 3.

Figure 3.20: Temperature transition during stage 1 vs. power supplied

Figure 3.21: Temperature transition during stage 5 vs. power supplied

The thickness of the glass substrate can also be variable from 100nm to 1mm. The

effect of the variable thickness on temperature transitions is illustrated in Figure 3.22 to 3.24. As shown in these figures, a thinner glass substrate results in a shorter transition time.

Figure 3.22: Temperature transition in stage 1 vs. substrate thickness

Figure 3.23: Temperature transition in stage 3 vs. substrate thickness

Figure 3.24: Temperature transition in stage 5 vs. substrate thickness

As a summary, the transition times are listed in Table 3.8, where data is obtained under the conditions of 0.25W heater power and 4m/s incoming air velocity.

Table 3.8: Summary of transition duration and rates of heating and cooling for the proposed PCR microchip

Stage	Duration	Rate of heat transfer
Stage 1 (Transition from extension to denaturation)	1.0244 sec	24.4°C/s
Stage 3 (Transition from denaturation to annealing)	3.5012 sec	11.4°C/s
Stage 5 (Transition from annealing to extension)	0.6147 sec	24.4°C/s

3.4.2 Analytical investigation of thermal cross-talk in PCR chamber array

According to the proposed chip design, the model of thermal cross-talk in PCR chamber array is illustrated as Figure 3.25.

Figure 3.25: Model of thermal cross-talk in PCR chamber array. where $T_{CM1(\text{with element } 2)}$ is the temperature of sample 1 with the presence of element 2, R_s is the thermal resistance between the heater and the bottom layer, R_c is the thermal resistance between the heater to the sample, R_{cx} is the thermal resistance between two adjacent elements, P_2 is the power of the second heater.

Based on the investigation in section 3.2.3, the temperature variation in sample 1 caused by the presence of PCR element 2 is

$$\Delta T_{CM1} = T_{CM1(\text{with chamber } 2)} - T_{CM1(\text{without chamber } 2)} = \frac{R_s(R_c + R_s)}{(R_{cx} + R_c + R_s)} P_2$$

Thermal conductivity of glass is $1.1 \text{ W}/(\text{m}\cdot\text{K})$. The distance between the heater and the bottom layer is 0.1 mm . The distance between the heater and the sample is 0.1 mm . The distance between the adjacent PCR elements is 9 mm . The maximum heating power is 0.25 W . Thus,

$$R_c = 0.909091 \times 10^{-4} \text{ m}^2 \text{ K} / \text{W}; R_s = 0.909091 \times 10^{-4} \text{ m}^2 \text{ K} / \text{W}; R_{cx} = 8.1818182 \times 10^{-3} \text{ m}^2 \text{ K} / \text{W}$$

$$\text{Therefore, } \Delta T_{CM1(\text{max})} = \frac{R_s(R_c + R_s)}{(R_{cx} + R_c + R_s)} P_2 = 0.03932^\circ \text{C},$$

which means that the maximum thermal cross-talk in the proposed PCR chamber array is only 0.03932°C , which shows that the SU-8 thermal insulation material is effective and the thermal cross-talk occurs during PCR process in PCR chamber array can be negligible. In the DNA melting analysis, the temperatures of all elements increase simultaneously at the same rates, therefore no thermal cross-talk exists.

3.4.3 Analytical investigation of precise DNA melting analysis

According to the Equation (3.2.5) in section 3.2.2, the sample convection ratio, r_c , becomes

$$\Delta T = 0.01^\circ\text{C}; Gr = 152.12; Pr = 2.21; Nu = 1.92; h = 647.3 \text{ W}/(\text{m}^2 \text{ K}); L = 4 \text{ mm}; l = 0.1 \text{ mm} ,$$

$$r_c = 0.6\pi K Pr^{\frac{1}{5}} g^{\frac{1}{5}} \beta^{\frac{1}{5}} \Delta T^{\frac{6}{5}} (L^{\frac{3}{5}} l) \left(\frac{1}{P} \right) \left(\frac{1}{v} \right)^{\frac{2}{5}} = 1.626 \times 10^{-5} = 0.001626\%$$

where ΔT is the difference between the initial temperature and the final temperature, Gr is Grashof Number, Pr is Prandtl Number, Nu is Rayleigh Number, h is the convection coefficient, L is the diameter of the sample, l is the thickness of the sample. So it can be assumed that only the thermal conduction is considered during the DNA melting analysis.

A cross-sectional view of the single PCR element is illustrated in Figure 3.26.

Figure 3.26: Cross-sectional view of single PCR element

Because the heater is close (0.1mm) to PCR sample, the temperature of the sample can be controlled almost directly. The PCR sample should be heated from a low temperature to a high temperature for DNA melting analysis. The temperature transition range is defined as Temperature Scan Interval (TSI), i.e. from 60°C to 100°C. The smallest temperature increment that could be controlled is defined as Temperature Scan Resolution (TSR). The Melting Transition Step (MTS), defined as one step of temperature increment, such as 0.01°C from 80°C to 80.01°C, over the entire range of temperature variation in a DNA melting analysis, can be controlled precisely. If the temperature difference between the top and bottom nodes of the sample is less than a critical value (10% of the temperature increment of the Melting Transition Step), it can be assumed the sample temperature is uniform. The most important parameter to describe the temperature variation in the DNA melting

analysis is the Melting Transition Time (MTT). The Melting Transition Time is defined as the time duration for both the top and bottom nodes of the sample to achieve 90% of the temperature increment in a Melting Transition Step. The Melting Transition Time not only illuminates the temperature uniformity, but also indicates how fast a temperature increment can be accomplished. Less Melting Transition Time is better for the DNA melting analysis.

Based on the precious investigation of heat conduction using the composite layers model (section 3.2.2), the parameters of the proposed microchip are listed in the following:

$$L_1 = 10^{-4} m; a^2 = 1.43 * 10^{-7} m^2 / s; L_2 = 10^{-4} m; b^2 = 6.98 * 10^{-7} m^2 / s; T_0 = 80^\circ C; T_1 = 80.01^\circ C$$

where L_1 is the thickness of sample, L_2 is the thickness of substrate; a^2 is the thermal diffusivity of sample, b^2 is the thermal diffusivity of substrate, T_0 is the initial temperature, T_1 is the final temperature.

According to Section 3.2.2, the temperatures of top node and bottom node of sample are $T_{top} = 80.01 - 0.01e^{-3.42t}$ and $T_{bottom} = 80.01 - 0.01e^{-7.22t}$, which are illustrated in Figure 3.27.

As illustrated in Figure 3.27, MTT for the proposed PCR microchip is 0.63 second.

As a conclusion, a precise temperature control at 0.01°C scan resolution in DNA melting analysis can be achieved by ramping up the temperature at 1°C/min.

Figure 3.27: Temperatures of bottom node and top node of sample vs. Time

3.5 Summary

According to above calculations and analyse on the proposed chip design, its capabilities of rapid thermal cycling (less than 8 second) in the PCR process, precise temperature control (0.01 °C temperature scan resolution) in the DNA melting process, and neglectable thermal cross-talk ($\pm 0.03932^{\circ}\text{C}$) in PCR chamber array were proven analytically feasible.

Chapter 4 Numerical simulation of on-chip heat transfer

4.1 Assumptions of models

In the present work, computer simulations by a commercial software package (ANSYS, version 6.1) is used to investigate assess the thermal performance of PCR microchip. In the numerical simulation, some simplifications are used to make the numerical models easier to construct. The microheater is simplified as a disk area with constant heat flux but no thickness (Appendix F). The property of the sample is simplified to those of water. In the mesh process during setting up the simulation model, the whole model is divided by $2\mu\text{m} \times 2\mu\text{m} \times 2\mu\text{m}$ cube for 3D model or $2\mu\text{m} \times 2\mu\text{m}$ square for 2D model, which are at least 40 times less than the minimal geometric characteristics in the structure.

4.2 Simulation of PCR process in microchip

Based on previous assumptions, a model of PCR element is set up in Figure 4.1. The labeled nodes in the PCR element are located strategically to investigate the temperature transition and uniformity during PCR process in the microchip.

Figure 4.1: Model of PCR element plotted using ANSYS (Version 6.1). Node 1 is located at the centre of Sample; node 2 is located at the centre of Sample top surface; node 3 is located at the centre of Sample bottom surface; node 4 is located at the boundary of Sample top surface; node 5 is located at the boundary of Sample bottom surface; node 6 is located at the center of heater surface.

As a simulation result, the temperature distribution of the PCR element at the end of stage 1 is illustrated in Figure 4.2. Obviously, most heat concentrates in the central region of the element, while the boundary area remains low temperature. Almost no thermal cross-talk occurs.

Figure 4.2: Temperature distribution of a single PCR element at the end of stage 1. The temperature of the central region of the element is almost uniform; and temperature of the boundary remains about 30°C lower than the central region.

Figure 4.3: Temperature variations of different nodes of Sample in stage 1. The thermal transition between extension (70°C) and denaturation (95°C) can be accomplished in 0.72 second. The heating rate of the centre node (node 1) in the Sample is 33.3°C/s .

As illustrated in Figure 4.3, the thermal transition between 70°C and 95°C can be accomplished in 0.72 second. The ramping rate of the centre node (node 1) in the Sample is 33.3°C/s . The center nodes ramp faster than the boundary nodes; and the bottom nodes ramp faster than the top nodes. The maximum temperature difference along the radius of Sample during stage 1 is 6°C , while the maximum temperature difference in thickness direction during stage 1 is 1.3°C .

In stage 3, only the convection load is applied in the PCR model. Figure 4.4 shows the temperature distribution in the PCR element at the end of stage 3. Figure 4.5 shows the temperature curves from 95°C to 55°C during stage 3. The cooling rate of centre node in Sample (node 1) is -8°C/s . Because the cooling area covers almost all surfaces of the model as shown in Figure 4.4, it takes longer time than heating. But the temperature differences between different nodes (center nodes and boundary nodes, top nodes and bottom nodes) are less than those in the heating processes. The maximum temperature difference in the Sample is 0.5°C in stage 3.

Figure 4.4: Temperature distribution of a single PCR element at the end of stage 3. The temperature of the central region of the element is uniform; and temperature of the boundary remains about 15°C lower than the central region.

Figure 4.5: Temperature variations of different nodes of Sample in stage 3. The thermal transition between denaturation (95°C) and annealing (55°C) can be accomplished in 5 second. The cooling rate of the centre node (node 1) in the sample is -8°C/s.

As shown in Figure 4.6, only the Sample is heated at the end of stage 5, while the boundary area remains at low temperature. Almost no thermal cross-talk occurs in such a short time (0.45 sec).

Figure 4.6: Temperature distribution of a single PCR element at the end of stage 5. The temperature of the central region of the element is almost uniform; and temperature of the boundary remains about 20°C lower than the central region.

Figure 4.7: Temperature variations of different nodes of Sample in stage 5. The thermal transition between annealing (55°C) and extension (70°C) can be accomplished in 0.45 second. The heating rate of the centre node (node 1) in the sample is also 33.3°C/s.

Similar to stage 1, a rapid heating rate (33.3°C/s) at node 1 is achieved during process 5, as shown in Figure 4.7. The PCR Sample can be heated from 55°C to 70°C in 0.45 second. But temperature uniformity is not good as cooling process. The maximum temperature difference along the radius of Sample during stage 5 is 4.5°C, while the maximum temperature difference across the thickness of sample during stage 5 is 1.1°C.

As a summary, the time durations and rates of temperature transitions obtained by simulations for the proposed microchip are listed in Table 4.1.

Table 4.1: Time durations and rates of temperature transitions (at node 1) obtained by simulations for the proposed microchip

Stages	Time	Rate
Temperature transition from extension process (70 °C) to denaturation process (95 °C)	0.72 sec	+33.3 °C/s

Temperature transition from denaturation process (95 °C) to annealing process (55 °C)	5 sec	- 8 °C/s
Temperature transition from annealing process (55 °C) to extension process (70 °C)	0.45 sec	+33.3 °C/s

Although the Sample boundary nodes (node 4 and 5) can not reach the constant temperatures at the end of thermal transitions (the target temperature (70°C, 94°C, 55°C) is set based on the temperature of node 1), as shown in Figure 4.8 they can do so after holding on the temperatures for 0.28 second, thus the duration of the steady stages, stage 2, 4 and 6, are set to 0.6 second, which are longer than 0.28 second. Based on the above simulation results at node 1, a complete PCR thermal cycle can be accomplished in 8 seconds as shown in Figure 4.8, which reduces the total time of a PCR process greatly. At this cycle time, a 30 cycle PCR can be accomplished in 4 minutes, and this may generate a very significant impact to molecular biology and clinical diagnostics.

Figure 4.8: Simulation result of a complete PCR thermal cycle (accomplished in 8 sec) labelled with PCR stages (defined at section 3.2.1, as shown in Figure 3.2)

4.3 Simulation of thermal cross-talk in PCR chamber array

Because the thermal condition of the PCR element is symmetrical about the center of Sample, a numerical model is set up as two adjacent half PCR elements with a common boundary, one is on the left half and another is on the right half, as shown in Figure 4.9. Simulation result of PCR chamber array transient response under alternate control, as the left half PCR element is heating while the right half PCR element is cooling (vice versa), is illustrated in Figure 4.10. This result corresponds to the worst case, i.e. a half PCR element is at the highest temperature during PCR and another is at the lowest temperature during PCR. The temperature distributions and the rate of heat transfer of adjacent half PCR elements in each PCR stage over the entire time duration of a PCR cycle (8 seconds) are illustrated in Figure 4.11 to 4.21.

Figure 4.9: Numerical model of thermal cross-talk in PCR chamber array. (a) Cross-sectional view of the PCR chip (b) Sketch of adjacent half PCR elements (only a half of PCR element is shown, due to symmetry); (c) Model sketch of thermal cross-talk in adjacent half PCR elements. Node A is located at the top surface of the common boundary; node B is located at the bottom surface of the common boundary; node C is located at the center node of the left Sample; node D is located at the center node of the right Sample. (d) 3D schematic diagram of adjacent half PCR elements. Interface A is comprised of the middle interface of left wall (the half circle curve surface) and two vertical interfaces, which is marked by the red color; Interface B is located at the middle vertical interface of common boundary, which is marked by the green color; Interface C is comprised of the middle interface of right wall (the half circle curve surface) and two vertical interfaces, which is marked by the blue color. (e) FEM mesh for studying the thermal cross-talk in adjacent half PCR elements plotted using ANSYS (Version 6.1).

Figure 4.10: Simulated temperature profile at node A, B, C, D within a PCR cycle (8 sec), labelled with PCR stages (defined at section 3.2.1, as shown in Figure 3.2) under alternative control. $\Delta T_{\max}$ represents the maximum of the temperature difference between the adjacent PCR elements. This temperature control in PCR chamber array corresponds to the worst case, i.e. a PCR element is at the highest temperature (node D) during PCR and another is at the lowest temperature during PCR (node C).

Figure 4.11: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 0.44 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 5, and the right half PCR element, represented by node D, is in stage 3).

Figure 4.12: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 0.7 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 6, and the right half PCR element, represented by node D, is in stage 3).

Figure 4.13: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 1.44 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 1, and the right half PCR element, represented by node D, is in stage 3).

Figure 4.14: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 1.7 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 2, and the right half PCR element, represented by node D, is in stage 3).

Figure 4.15: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 6 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 3, and the right half PCR element, represented by node D, is in stage 4).

Figure 4.16: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 6.3 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 3, and the right half PCR element, represented by node D, is in stage 5).

Figure 4.17: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 6.74 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 3, and the right half PCR element, represented by node D, is in stage 6).

Figure 4.18: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 7 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 3, and the right half PCR element, represented by node D, is in stage 1).

Figure 4.19: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 7.7 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 4, and the right half PCR element, represented by node D, is in stage 1).

Figure 4.20: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 7.74 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 4, and the right half PCR element, represented by node D, is in stage 2).

Figure 4.21: Temperature distribution (a) and the rate of heat transfer (b) across adjacent half PCR elements at 8 second in a PCR cycle (the left half PCR element, represented by node C, is in stage 4, and the right half PCR element, represented by node D, is in stage 2). The thermal properties at 8

second are same with those at 0 second. The 8 second moment is the end of the one PCR cycle, and also the beginning of the next PCR cycle.

As shown in Figure 4.11 to 4.21, the common boundary always has the lowest temperature through out a PCR cycle (the left images); and the rate of heat transfer at the SU-8 wall, which is represented by interface A and C (the right images), is much higher than that of the common boundary, which is represented by interface B (the right images). Due to the adoption of the convection channels, the heat transfer from hot PCR element to cold PCR element is mainly isolated by the air flow. Thus there is almost no thermal cross-talk between adjacent PCR elements within the time frame for the PCR process. The temperature transition during PCR is too rapid for a thermal cross-talk to occur through the bridging structures (common boundary) linking two adjacent PCR elements.

4.4 Simulation of high resolution DNA melting analysis

High resolution DNA melting analysis is a slow and precise temperature scan process from a low temperature (60°C) to a high temperature (95°C), investigating the DNA melting temperature. The resolution of a DNA melting analysis is determined by the precision of temperature control. To achieve high resolution detection in DNA melting analysis, we explore a temperature scan resolution of 0.01°C. The numerical model for the DNA melting analysis is illustrated in Figure 4.22.

Figure 4.22: Numerical model of high resolution DNA melting analysis. (a) Cross sectional view of a single PCR element. Node 1 is located at the centre of Sample bottom surface; node 2 is located at the centre of Sample top surface; node 3 is located at the boundary of Sample bottom surface; node 4 is located at the boundary of Sample top surface; node 5 is located at the center of the heater (the temperature of the heater is assumed to be uniform). (b) FEM model of the DNA melting analysis, plotted using ANSYS (Version 6.1)

Figure 4.23: Temperature variation within a DNA melting step from 80°C to 80.01°C

Figure 4.24: Temperature distribution within a DNA melting step from 80°C to 80.01°C

Figure 4.25: Temperature variation within a DNA melting step from 60°C to 60.01°C

Figure 4.26: Temperature variation within a DNA melting step from 100°C to 100.01°C

Because the heater is close to the sample (0.01mm) and the DNA sample is shallow (0.008mm), the heat from heater can transfer efficiently to the DNA sample. As shown in Figure 4.23, 4.25 and 4.26, the Melting Transition Times (defined in section 3.4.3) are almost same (0.5 second). Due to the temperature variations at 60° C, 80° C, and 100 ° C are similar, they are assumed to represent each Melting Transition Step. As illustrated in Figure 4.24, the temperature distribution in the sample is almost uniform (The maximum temperature difference in the sample is less than 0.001° C). As a conclusion, the temperature uniformity and control precision of the proposed microchip during DNA melting analysis could be guaranteed with a temperature ramping rate of less than 1.2° C/min (0.02° C/s).

4.5 Simulation of effect of PCR element characteristics on PCR cycling speed

Different characteristics of the PCR element (such as the thickness of substrate or Sample, and the thermal conductivity of substrate) will affect PCR speed (heating/cooling rate). Following investigations are based on the temperatures of different nodes in the Sample, as shown in Figure 4.27.

Figure 4.27: A cross-sectional view of a single PCR element. Node TC is located at the centre of Sample top surface; node BC is located at the centre of Sample bottom surface; node C is located at the centre of Sample; node TB is located at the boundary of Sample top surface; node BB is located at the boundary of Sample bottom surface.

4.5.1 Effect of PCR element geometry

As the first parameter to study, the thickness of the substrate is set as a variable for the investigation of PCR cycling speed.

(a) In heating period.

Figure 4.28: Heating rates at different nodes vs. thickness of substrate

Figure 4.29: Heating rate differences vs. thickness of substrate

As shown in Figure 4.28, the heating rates decrease when the thickness of the substrate increases. The maximum rate is 37°C/s at the centre node on the bottom surface of the Sample. As shown in Figure 4.29, the heating rate differences (defined as the absolute difference between two heating rates) between the center node and boundary nodes of the Sample decrease when the thickness increases. The maximum heating rate difference across the thickness of Sample cylinder is 4°C/s , while the

maximum heating rate difference along the radius of Sample cylinder is 10°C/s . To Balance the heating rate and the heating rate difference, the recommended range of substrate thickness is below 0.2mm to achieve an optimal PCR condition.

(b) In cooling period.

As shown in Figure 4.30, the cooling rates decrease when the thickness of substrate increases; the maximum is 11.5°C/s . But the cooling rate differences (defined as the absolute difference between two cooling rates) between different nodes (Figure 4.31) almost keep at a constant value, while the maximum is 0.9°C/s . Thus, to achieve a high cooling speed, the range of substrate thickness below 0.12mm is recommended.

Figure 4.30: Cooling rates at different nodes vs. thickness of substrate

Figure 4.31: Cooling rate differences vs. thickness of substrate

As the second parameter to study, the thickness of the sample is set as a variable for the investigation.

(a) In heating period.

Figure 4.32: Heating rates at different nodes vs. thickness of sample

Figure 4.33: Heating rate differences vs. thickness of sample

As shown in Figure 4.32, the heating rates decrease when the thickness of the Sample increases. As illustrated in Figure 4.33, heating rate differences along the radius of Sample decrease when the thickness increases, but the heating rate differences across the thickness of Sample increase simultaneously. To balance these effects, the thickness range of the Sample cylinder from 0.08mm to 0.15mm is recommended.

(b) In cooling period.

Figure 4.34: Cooling rates at different nodes vs. thickness of sample

Figure 4.35: Cooling rate differences vs. thickness of sample

As shown in Figure 4.34, the cooling rates decrease when the thickness of Sample increases, and the maximum is 13°C/s . But the cooling rate differences (Figure 4.35) increase too. Thus, the thickness of the Sample less than 0.12mm is recommended.

4.5.2 Effect of thermal conductivity of substrate

The thermal conductivity of the substrate is set as a variable for the investigation.

(a) In heating period.

Figure 4.36: Heating rates at different nodes vs. thermal conductivity of substrate

Figure 4.37: Heating rate differences vs. thermal conductivity of substrate

A peak (36°C/s) at $0.8\text{W}/(\text{m}\cdot\text{K})$ is shown in Figure 4.36. As illustrated in Figure 4.37, the heating rate differences reach their maximums (9.5°C/s heating rate difference along the radius of Sample cylinder, 2°C/s heating rate difference across the thickness of Sample cylinder) at $0.9\text{W}/(\text{m}\cdot\text{K})$. Thus the thermal conductivity of substrate from 0.4 to $3\text{W}/(\text{m}\cdot\text{K})$ is recommended.

(b) In cooling period.

Figure 4.38: Cooling rates at different nodes vs. thermal conductivity of substrate

Figure 4.39: Cooling rate differences vs. thermal conductivity of substrate

As shown in Figure 4.38, the cooling rates approach to 11°C/s when the thermal conductivity of the substrate increases. As illustrated in Figure 4.39, the cooling rate differences along the radius of Sample reach a peak (0.85°C/s) at $0.8\text{W}/(\text{m}\cdot\text{K})$, while the cooling rate differences across the thickness of Sample always keep at a low level ($<0.03^{\circ}\text{C/s}$). Thus the recommended range for the thermal conductivity of the substrate is larger than $1\text{W}/(\text{m}\cdot\text{K})$.

4.6 Simulation of effect of PCR element characteristics on thermal cross-talk in PCR chamber array

Different characteristics of the PCR element can also affect the thermal cross-talk in PCR chamber array. In this simulation, the data of heating rate and cooling rate are all collected at the node 1 in Figure 4.1. The heating/cooling rate with thermal cross-talk is detected from the adjacent PCR elements with alternate control (the temperature profiles are shown in Figure 4.10). The heating/cooling rate without thermal cross-talk is detected from single PCR element (the temperature profile is shown in Figure 4.8).

4.6.1 Effect of PCR element geometry

As the first parameter to study, the thickness of the substrate is set as a variable for the investigation of thermal cross-talk.

Figure 4.40: Heating rate with/without thermal cross-talk vs. thickness of substrate

Figure 4.41: Cooling rate with/without thermal cross-talk vs. thickness of substrate

Figure 4.40 shows two curves with a similar shape except in the region of thin substrate which means that a good thermal insulation can be guaranteed when the substrate is thicker than 0.08mm. Almost the same conclusion is obtained by the simulation on the cooling period (Figure 4.41).

As the second parameter to study, the thickness of the sample is set as a variable for the investigation.

Figure 4.42: Heating rate with/without thermal cross-talk vs. thickness of sample

Figure 4.43: Cooling rate with/without thermal cross-talk vs. thickness of sample

Figure 4.42 and 4.43 show the negligible effect of the thermal cross-talk. No matter how thick the Sample is, PCR speeds will not be affected in PCR chamber array.

4.6.2 Effect of thermal conductivity of substrate

The thermal conductivity of the substrate is set as a variable for the investigation.

Figure 4.44: Heating rate with/without thermal cross-talk vs. thermal conductivity of substrate

Figure 4.45: Cooling rate with/without thermal cross-talk vs. thermal conductivity of substrate

As shown in Figure 4.44, the heating rate in chamber array is more rapid than the heating rate in the single PCR element. When the thermal conductivity of substrate is more than 1.5 W/(m·K), a thermal cross-talk is obvious. Similar to the heating curves, as shown in Figure 4.45, the cooling rate difference of with/without thermal cross-talk increases greatly when the thermal conductivity of the substrate is more than 2W/(m·K). So the thermal conductivity range of less than 1.5W/(m·K) is recommended.

4.7 Simulation of effect of PCR element characteristics on precise temperature control during DNA melting analysis

Different characteristics of the PCR element can also affect the precise temperature control during DNA melting curve analysis. The Melting Transition Time (defined in section 3.4.3) is the most important criteria to evaluate the thermal conditioning during this process. Less Melting Transition Time can provide more precise temperature scan resolution and is better for the DNA melting analysis.

4.7.1 Effect of PCR element geometry

Figure 4.46 shows the effect of the thickness of the substrate on the Melting Transition Time. A substrate thicker than 0.17mm causes a “thermal delay”, which is defined as that the Melting Transition Time is more than 1 second. Thus, the recommended range for the thickness of substrate is below 0.15mm.

Figure 4.46: (a) Melting Transition Time vs. thickness of substrate (0.01 ~ 1.0mm); (b) Enlarged view of Melting Transition Time vs. thickness of substrate (0.01 ~ 0.2mm)

Figure 4.47 shows the effect of the thickness of sample on the Melting Transition Time. A Sample thicker than 0.2mm causes an obvious thermal delay (the Melting Transition Time is more than 1 second). So the recommended range for the thickness of Sample is less than 0.2mm.

Figure 4.47: (a) Melting Transition Time vs. thickness of sample (0.01 ~ 1.0mm); (b) Enlarged view of Melting Transition Time vs. thickness of sample (0.01 ~ 0.4mm)

4.7.2 Effect of thermal conductivity of substrate

Figure 4.48 shows the effect of the thermal conductivity of substrate on the Melting Transition Time. The Melting Transition Time approaches to infinity, when the thermal conductivity of substrate is less than 0.8 W/(m·K). So, the recommended range is larger than 0.8 W/(m·K).

Figure 4.48: Melting Transition Time vs. thermal conductivity of substrate. The Melting Transition Time for glass with 1.13 W/(m·K) thermal conductivity is 0.5 second; the Melting Transition Time for SU-8 with 0.2 W/(m·K) thermal conductivity is 3.2 second; the Melting Transition Time for SU-8 with 0.17 W/(m·K) thermal conductivity is 3.27 second.

4.8 Summary and discussion

As illustrated in Figures 4.1 to 4.8, the proposed PCR microchip is capable of achieving rapid thermal cycling. The heating rate reaches 33.3°C/s , while the cooling rate reaches 8°C/s . One complete PCR thermal cycle could be accomplished in 8 seconds.

The simulation results on the thermal cross-talk in the PCR chamber array verify that the thermal insulation assumption is valid. The temperature distribution in adjacent half PCR elements during thermal cycling shows that there is almost no heat transfer happened between adjacent elements within the PCR cycle time, as shown in Figure 4.9 to 4.21. Every PCR element could be controlled independently in chamber array.

As shown in Figure 4.22 to 4.26, the precise temperature control can be guaranteed with the ramping rate at 1.2°C/min during the DNA melting analysis.

As shown in Figure 4.27 to 4.48, the effects of PCR element characteristics, including the thickness of the substrate and the Sample, and the thermal conductivity of the substrate, on

- PCR thermal cycling speed
- PCR temperature uniformities
- PCR speed affected by thermal cross-talk in chamber array
- precise temperature control during DNA melting analysis

were simulated to optimize the parameters of the chamber. As a conclusion, these recommended parameter ranges are listed in Table 4.2 as a guideline for microchip design.

Table 4.2: Parameter ranges of PCR element characteristics for microchip design

Characteristic of PCR element	Range for microchip design
-------------------------------	----------------------------

Thickness of substrate (mm)	0.08 -- -- 0.1
Thickness of Sample (mm)	0.08 -- -- 0.12
Thermal conductivity of substrate (W/(m·K))	0.8 -- -- 1.5

Chapter 5 Microfabrication

5.1 Introduction

Micro-electro-mechanical-systems (MEMS) technology provides a microfabricate process platform to fabricate bio-chips on which many experiments are conducted in micro-scales or nano-scales. The micromachining methods used in MEMS technology have become the essential tools for the fabrication of various micro genetic diagnostic devices, such as PCR microsystems for DNA amplification and identification, biochips for detection of hazardous chemical and biological agents, and microsystems for high-throughput drug screening. The MEMS technology has presented the possibility to conduct genetic analysis assays with high efficiency and relatively low costs. There are three basic building blocks in the MEMS technology, namely deposition processes, photolithography, and etching processes [47, 76].

5.2 Deposition Processes

One of the basic building blocks in MEMS processing is the ability to deposit thin films of materials. The MEMS deposition technology can be classified in two groups:

5.2.1 Depositions due to a chemical reaction

These include,

- Chemical Vapour Deposition (CVD)
- Electrodeposition
- Epitaxy
- Thermal oxidation

These processes exploit the generation of solid materials directly from chemical reactions in gas and/or liquid compositions or with the substrate material. The solid

material is usually not the only product formed by the reaction. Byproducts can include gases, liquids and even other solids.

5.2.2 Depositions due to a physical reaction

These include,

- Physical Vapour Deposition (PVD)
- Casting

In these processes, the material deposited is physically bonded onto the substrate.

5.2.3 Selection of deposition processes

Among the above methods, the sputtering and the casting are selected in this work.

5.2.3.1 Sputtering

Sputtering is a technology by which material is released from the source at much lower temperature than evaporation. The substrate is placed in a vacuum chamber with source material, known as target, and an inert gas introduced at low pressure. Gas plasma is struck using an RF power source, causing gas to become ionized. Ions are accelerated towards the surface of the target. The basic principle of sputtering is same as evaporation. The differences typically are related to the manner in which the ion bombardment of target is realized [78].

5.2.3.2 Casting

In this process, deposit material is dissolved in liquid form in a solvent. Material can be loaded to substrate by spraying or spinning. Once the solvent is evaporated, a thin film of the material remains on substrate. This is particularly useful for polymer materials, which may be easily dissolved in organic solvents, and it is the common method used to apply photoresist to substrates (in photolithography). The thickness that can be cast on a substrate can vary from a single monolayer of molecules

(adhesion promotion) to tens of micrometers. The control on film thickness depends on exact conditions, but can be sustained within +/-10% in a wide range [78].

5.3 Photolithography

Photolithography is typically the transfer of a pattern to a photosensitive material by selective exposure to a radiation source such as UV light. In lithography for micromachining, the photosensitive material used is photoresist. When the photoresist is exposed to a radiation source of a specific wavelength, the chemical resistance in solution changes. If the exposed material is etched away by developer and unexposed region remains, the material is considered to be a positive resist (such as AZ 7220). Conversely, it is considered to be a negative resist (such as SU-8). After lithography, the photosensitive layer can be used as a temporary mask to transfer pattern to the underlying layer or to deposit. After deposition, the resist is subsequently etched away, and material deposited on the resist is "lifted off".

The lithography process steps can be characterized in the following sequence.

- Substrate Pretreatment

To obtain the maximum process reliability, substrates should be clean and dry by a solvent cleaning or a rinse with dilute acid, followed by a DI water rinse. Where applicable, substrates are subjected to a piranha etch / clean (H_2SO_4 & H_2O_2).

- Resist spin/spray

Coat the wafer with photoresist either by spinning or spraying. A uniform coating is desired. The spin conditions for different photoresists are listed in Table 5.1:

Table 5.1: Spin conditions for different photoresists

Resist	SU-8 5	SU-8 100	AZ 7220
Thickness (μ ms)	5	200	2

Spin speed (rpm)	3000	1500	1000
Time (Sec)	30	20	20

- Soft bake

After the resist is applied to substrate, it must be soft baked to evaporate the solvent and densify the film. The soft bake conditions are listed in Table 5.2.

Table 5.2: Soft bake conditions for different photoresists

Resist	Thickness	Bake
SU-8 5	5(μ ms)	1min@65° C, 3mins@95° C
SU-8 100	200(μ ms)	20mins@65° C, 50mins@95° C
AZ 7220	2(μ ms)	1min@100° C

- Exposure

The resist is exposed in a UV aligner (OAI J500-IR) with an intensity of 10mW/cm² at the wavelength of 365nm. The exposure times are listed in Table 5.3:

Table 5.3: Exposure times for different photoresists

Resist	SU-8 5	SU-8 100	AZ 7220
Time (Sec)	7	50	10

- Post exposure bake

Following the exposure, a post exposure bake (PEB) must be performed to selectively cross-link the exposed portions of the film. The bake conditions are listed in Table 5.4.

Table 5.4: Post bake conditions for different photoresists

Resist	Thickness	Bake
SU-8 5	5(μ ms)	1min@65° C, 1min@95° C

SU-8 100	200(μ ms)	1min@65° C, 16mins@95° C
AZ 7220	2(μ ms)	1min@110° C

- Develop

A development is the selective removal of resist after exposure (exposed resist if resist is positive, unexposed resist if resist is negative). The development conditions are listed in Table 5.5.

Table 5.5: Development conditions for different photoresists

Resist	Thickness	Develop time
SU-8 5	5(μ ms)	1min
SU-8 100	200(μ ms)	18mins
AZ 7220	2(μ ms)	2mins

- Hard bake

The resist may be ramp/step hard baked between 150-200°C on a hot plate or in a convection oven to further cross link the resist material.

- Descum

The removal of resist scum that may occlude open regions in pattern is necessary [77].

5.4 Etching processes

In order to form a functional MEMS structure on a substrate, it is necessary to etch the thin films previously deposited and/or the substrate itself. In general, there are two classes of etching processes: wet etching (the material is dissolved when immersed in a chemical solution) and dry etching (the material is sputtered or dissolved by reactive ions or a vapor phase etchant).

5.4.1 Wet etching

This is the simplest etching technology. All it requires is a container with a liquid solution that will dissolve the material in question. The mask will not be dissolved or at least etched much slower than the material to be patterned. Wet etching works well for etching thin films on substrates and/or the substrate itself. The etching conditions of glass, Gold, Copper, and Chromium are listed in Table 5.6.

Table 5.6: Wet etching conditions for different materials [78]

Application/material	Etchant	Remark/reference
Glass (SiO ₂)	HF:NH ₄ F (buffered HF 28ml HF, 170ml H ₂ O, 113g NH ₄ F)	Etch rate is 100 – 500 nm/min at 25 °C
Gold	4g KI, 1gI ₂ , and 40ml H ₂ O	0.5 - 1 μ m/min
chromium	1 ml HCl, 1ml glycerine	80nm/min
Copper	FeCl ₃	0.05 – 0.1 μ m/min

5.4.2 Dry etching

Dry etching is expensive to run as compared to wet etching. However, if feature resolution in thin film structures or vertical sidewalls for deep etchings in the substrate is of primary concern, then dry etching should be considered. The dry etching technology can split in three separate classes called reactive ion etching (RIE), sputter etching, and vapor phase etching [78].

5.5 Chip fabrication processes

The fabrication process of the proposed PCR microchip includes:

- Sputtering
- Photolithography
- Wet etching

The fabrication process of PCR microchip is described in the following. All figures depict the cross sectional view of the microchip at different sections, and steps of the chip microfabrication.

5.5.1 Fabrication process of PCR chamber array chip

To illustrate clearly, the middle layer of one single PCR element (designed in Section 3.3.1.2) in the PCR chamber array chip is shown in Figure 5.1. The microfabrication process of PCR chamber array chip can be described as the following:

1. A glass slide was used as the substrate after standard cleaning and drying processes.
2. A layer ($200\text{ \AA}$) of Cr and a layer ($1000\text{ \AA}$) of Gold were coated on the surface of glass substrate.
3. A layer ($2\mu\text{m}$) of AZ 7220 photoresist is spin-coated on the Au surface.
4. The AZ 7220 photoresist was photolithographically patterned by a mask under UV light.
5. The unexposed AZ 7220 was removed by the AZ 7220 developer.
6. The unprotected Au and Cr were removed by an Au/Cr etchant.
7. The exposed AZ 7220 was removed by Acetone.
8. A layer ($200\mu\text{m}$) of SU-8 100 was spin-coated on the reversed surface of the glass slide.
9. The SU-8 photoresist was exposed by a photomask under UV light.
10. The unexposed SU-8 100 resist was removed by the SU-8 developer to form the PCR chamber wall and boundary on the glass slide.

11. The reversed surface of the glass slide without protection was etched by the HF etchant.
12. The Au and Cr sacrificial layer was removed by the Au/Cr etchant.

Figure 5.1: Fabrication process for the PCR chamber array chip

5.5.2 Fabrication process of microheater and temperature sensor chip

The next, the bottom layer with micro heater and the sensor (designed in Section 3.3.1.3) were fabricated using the following processes as illustrated in Figure 5.2:

1. A glass slide was used as the substrate after standard cleaning and drying processes.
2. A layer of protecting Au/Cr was coated by sputtering and a layer (2 μ m) of the AZ 7220 photoresist was spin-coated on the glass slide
3. The AZ 7220 photoresist was exposed.
4. The exposed AZ 7220 resist was removed by the AZ 7220 developer.
5. The unprotected Au/Cr was etched by the Au/Cr etchant and the AZ 7220 layer was removed by Acetone.
6. A layer (1 μ m) of Copper was coated on the glass surface by sputtering, and a layer (2 μ m) of the AZ 7220 photoresist was spin-coated on the surface of the copper layer.
7. The AZ 7220 resist was exposed by UV light and developed, and the unprotected Cu layer was etched to obtain the leads of the sensor.
8. A layer of a 5 μ m thick SU-8 5 photoresist was spin-coated on the glass surface and exposed with a photomask as an insulating layer
9. A layer of a 1 μ m thick copper and a layer of a 2 μ m thick AZ 7220 photoresist was coated on the surface of the exposed SU-8 5.
10. The layer of Copper was patterned as the leads of the heater.
11. A layer (2 μ m) of the AZ 7220 photoresist was spin-coated.

12. The layer of the AZ 7220 resist was exposed and developed to obtain necessary patterns.
13. A layer ($400 \text{ \AA}$) of Pt was coated by sputtering on the surface of the exposed AZ 7220.
14. The micro heater and the sensor patterns were fabricated by the “lift-off” process.
15. The reversed surface of the glass slide without protection was etched by the HF etchant to form the convection channel, and after that, the protect Au/Cr layer was removed.

Chapter 5 Fabrication

Figure 5.2: Fabrication process for the microheater and temperature sensor chip

The middle layer is bounded to the bottom layer using PDMS to glass bonding. A thin layer of PDMS (less than $5\mu\text{m}$) is applied to the bottom layer and the bonding of the two layers is formed by applying pressure to them and curing in a 60°C oven. The PDMS to glass bonding is reversible and the bottom layer can be reused. Based on above MEMS technology and microfabrication processes, a single PCR element with bonded the bottom layer and middle layer was fabricated and shown in Figure 5.3. To indicate the size of the chamber, a one Singapore dollar coin was placed next to the single PCR element.

Figure 5.3: Photo of the bonded bottom layer and middle layer with a Singapore dollar coin. This PCR element includes glass substrate (transparent), SU-8 boundary and wall, convection channel, leads, thin film temperature sensor and thin film heater (in the enlarged image).

The fabrication of the top layer is similar with that of the middle layer. A $120\mu\text{m}$ SU-8 50 layer is spun onto the 0.1mm thick glass slide, soft-baked and then patterned to form the cover, instead of the wall and boundary, using photolithography. The top layer is bonded to the middle layer using PDMS to SU-8 bonding. The PDMS to SU-8 bonding is reversible and the chamber can be filled with PCR sample by removing the top layer. The sample loading and sealing of the PCR chambers will be carried out at the chip operation stage and described in Section 6.5.2.

5.6 Calibration of RTD thin film temperature sensor

Before an actual temperature measurement is taken, the thin film temperature sensor should be calibrated to guarantee the accuracy of readings. The RTD thin film temperature sensor was placed into water/ice mixture (0°C) and boiling water (100°C) for a preliminary calibration (The relation between the platinum resistance of the sensor and the temperature is linear [71]). The line passing through the ice point and the boiling point, as shown in Figure 5.4, was used as the calibration curve.

Figure 5.4: RTD sensor calibration curve, where R is the RTD output resistance and T is the actual temperature.

Chapter 6 Experiments and results

6.1 Reference PCR system I: Air flow thermal cycling

6.1.1 Air flow PCR system setup

Based on commercial PCR devices, an air flow PCR machine was set up as a reference for our chip PCR system. The reason to develop reference PCR systems is that most existing PCR microchip are operated based on either air heating and cooling or thermoelectric heating and cooling principle. Additionally, these two reference systems are able to provide a good profile to demonstrate the difference between all PCR microchip and the chips used in the conventional systems. Driven by two electrical fans, heating/cooling air was guided to flow through the PCR chip by convection channels to realize thermal cycling (Figure 6.1 a and b). A thermocouple temperature sensor was placed below the PCR chip (Figure 6.1 c).

Figure 6.1: Airflow and convection channels in air PCR system (a) Top view of airflow in convection channels during heating stage (The channel gates are closed); (b) Top view of airflow in convection channels during cooling stage (The channel gates are open); (c) A cross-sectional view of airflow passing through the chip, where the thermocouple is located below the PCR chip.

A PID temperature controller (WATLOW Control series 982) was used to adjust the power input to the heater [79]. Major specifications of the PID temperature controller are listed in Table 6.1.

Table 6.1: Specifications of PID temperature controller, WATLOW Control series 982 [80].

Calibration accuracy and sensor conformity:	$\pm 0.1\%$ of span
Temperature stability:	$\pm 0.1^{\circ}\text{C}/^{\circ}\text{C}$
Input Range:	-73.3 to 537.7°C
Sample/Update Rates:	PID: 10Hz.
RTD resolution:	0.1°C

For the fluorescence detection of the PCR product, a Photometrics CoolSNAPcf camera from Roper Scientific was used. A megapixel sensor with small, square photosensing elements ensures that the image of each PCR chamber on the chip shows a sufficient spatial resolution. This feature, along with a high-speed digitizer, shutterless operation, and an interline CCD, makes CoolSNAPcf camera ideal for high-resolution biochip imaging. The image data are processed into digital signals by a PCI Card before transfer to a PC [81].

Based on above devices, the working principle of the air flow PCR system is illustrated in Figure 6.2. The PID controller receives temperature signal using a thermocouple and transfers it to a PC. Conversely, the PC sends commands back to control the heater and the fans with PID method. At the same time, the CCD camera takes the fluorescence detection images and transfers them to a PCI card. After signal processing, the digital data are stored into a PC for further analysis.

Figure 6.2: Working principle of air PCR system

6.1.2 Experiment result from air flow PCR system

According to the experiments with air flow PCR system, a temperature curve of thermal cycling is illustrated in Figure 6.3.

Figure 6.3: Temperature curve of thermal cycling using the air PCR system

As shown in Figure 6.3, the heating rate was 6.7°C/s and the cooling rate was 0.8°C/s . Obviously, the air flow PCR system held temperature for a long time at every PCR stage. Data achieved were fluctuant, and overshoots occurred.

6.1.3 Discussion

Because the air flow PCR system needs to heat up/cool down air instead of the PCR chamber directly, heating/cooling rate could not be rapid. In addition, the fluctuation

of the airflow seems to be the reason for the fluctuant data and overshoots in the temperature curve. Another disadvantage of the air flow PCR system is the location of thermocouple which is far away from the PCR chamber and its temperature cannot accurately represent the sample temperature. It was found that the biggest difference between the thermocouple temperature and the sample temperature was 8°C, which resulted in a thermal delay and temperature control difficulties in the air flow PCR system.

6.2 Reference PCR system II: TEC thermal cycling

6.2.1 TEC PCR system setup

Thermoelectric coolers (TECs) are solid-state heat pumps used in applications such as temperature stabilization, temperature cycling, or cooling below ambient. Thermoelectrics can be used to heat or to cool, depending on the direction of the current. Using a thermoelectric in the heating mode is efficient because all its internal heating and the load from the cold side is pumped to the hot side. This reduces the power needed to achieve the desired heating [82, 83, 84].

Instead of expensive optical lens for photometric camera, a self-scanning photodiode array (NMOS linear image sensor array S3901-1024Q, HAMAMATSU) is selected in TEC system for fluorescence detection. It is designed specifically as detectors for multichannel spectroscopy. Each photodiode has a large active area, high UV sensitivity yet very low noise, delivering a high S/N even at low light levels [85, 86]. The image data must be processed into a digital signal by a data processing unit (HAMAMATSU C8799) before transferring to a PC.

Using the above devices, the working principle of the TEC system is illustrated in Figure 6.4. A thermocouple is fixed on the TEC surface (Figure 6.5). Similar with air PCR system, the temperature data and image data is transferred into PC, then PC send commands to control TEC (a positive input voltage for heating, while a negative input voltage for cooling).

Figure 6.4: Working principle of TEC PCR system

Figure 6.5: A side view of TEC PCR machine, where the thermocouple and the PCR chip are fixed on the TEC surface.

6.2.2 Experiment result from TEC PCR system

The experimental results from the TEC PCR system show a temperature curve of thermal cycling in Figure 6.6. The heating rate is 4.5°C/s and the cooling rate is 1.3°C/s . Overshoots were not obvious in the TEC system. But there are still a few

second waiting period at each PCR cycle, although it is shorter than the air PCR system.

Figure 6.6: Temperature curve of thermal cycling using the TEC PCR system

6.2.3 Discussion

Similar to the air flow PCR system, the thermocouple fixed on the TEC surface could not detect the accurate sample temperature. So it must cost a period of time to hold on at every PCR stage for temperature uniformity. Since the temperatures at different locations on a TEC surface are the same, it is difficult to realize different thermal cycling profile controls in different PCR chambers of a PCR array chip.

6.3 Chip PCR system

As mentioned in Chapter 2, the micro PCR chip (shown in Figure 6.7 and 6.8) can achieve faster rates of heating and cooling than the air PCR system and TEC PCR system. Better thermal properties can be obtained from this chip PCR system. To evaluate the design and simulation results, the chip PCR system is set up and investigated in the following sections.

6.3.1 Chip PCR system setup

After the chip design and fabrication, the chip PCR system can be setup as shown in Figure 6.7. A thin film RTD sensor is microfabricated onto the same layer of the microheater underneath the PCR sample. (Figure 6.8) The working principle of the chip PCR system is similar to the reference systems (Air PCR system and TEC PCR system). In the expected PCR microchip, a microheater, a convection channel and a RTD sensor are integrated.

Figure 6.7: Working principle of chip PCR system

Figure 6.8: A cross-sectional view of PCR chip, where the RTD sensor is deposited at the same layer of microheater. The thickness of top layer is $100\mu\text{m}$; the thickness of cover is $100\mu\text{m}$; the thickness of PCR sample is $80\mu\text{m}$; the thickness of substrate is $100\mu\text{m}$; the thickness of bottom layer is $100\mu\text{m}$.

6.3.2 Modules of chip PCR system

This module comprises of the heating and cooling elements for PCR rapid thermocycling and the fluorescence detection/imaging system. The photograph of this module is shown in Figure 6.9.

Figure 6.9: (a) Photographs of chip PCR system module; (b) Closed up view of the chip

The main components of chip PCR system are identified in above photograph. But the detail components integrated in the optics system, such as excitation source, excitation wavelength filter, detection wavelength filter are obstructed and are not presented in the photograph.

The descriptions of these components are listed as following.

CCD camera for high resolution fluorescence detection

DTA DISCOVERY DS260E camera is marked by its high sensitivity, reduced weight and dimensions. Kodak KAF CCD, electromechanical shutter, Peltier cooling system, electronics are integrated in this camera module. The camera is connected to the PC by means of a bi-directional parallel port. Major specifications of the DTA camera are listed in Table 6.2.

Table 6.2: Specifications of DTA DISCOVERY DS260E camera. [87]

CCD	KAF-0261E
Array format (H×V)	512×512
Pixel (H×V) μm	20×20
Size (H×V) mm	10.2×10.2
Fill factor %	100

Excitation wavelength filter

Sybr-Green dye is used in the experimentations for the detection of successful amplification of PCR products. The excitation wavelength for Sybr-Green dye is about 492nm. Therefore, a narrow band-pass interference filter from Edmund Industrial Optics [88] of 488nm is used. The use of the filter also allows the flexibility in the usage of other commercially available PCR fluorescent dyes, such as FAM, Tet, Tamra and ROX. Their excitation wavelengths are 470nm, 510nm, 540, and 547nm respectively [89]. The filters are interchangeable to suit the different excitation needs.

Detection wavelength filter

The detection wavelength for fluorescence emission due to Sybr-Green dye is 521nm. In this case, a narrow band-pass interference filter from Edmund Industrial Optics [88] of 520nm is used. Even though the Sybr-Green dye emits fluorescence wavelength of about 521nm, there are interferences from other light sources such as light of the excitation wavelength. Hence, the filter will remove light of unwanted wavelengths and greatly improves the specificity of the detection of the intensity of fluorescence emission from the PCR products. As mentioned before, filters allow the flexibility in the usage of other commercially available PCR fluorescent dyes, such as

FAM, Tet, Tamra and ROX. The filters are interchangeable to suit the different detection needs.

6.3.3 PID optimization

PID (Proportional, Integral, and Derivative) compensation is one of the most common forms of closed-loop control. Basic feedback control is the simplest configuration, and involves only a measurement, the PID calculation and an output. [90] The classical PID control equation is defined as

$$CO = K_p E + K_i \int_0^t E dt + K_d \frac{dE}{dt}$$

Where,

CO = Controller Output

K_p = Proportional Constant

K_i = Integral Constant

K_d = Differential Constant

t = Time.

And the error is defined as

E = (Process Variable – Set Point) (direct action)

Or

E = (Set Point – Process Variable) (reverse action)

In a direct acting control loop, an increase in the process measurement causes increase in the controller output.

PID control is crucial for attaining rapid thermocycling with minimal temperature fluctuations and overshoots/undershoots. Thus, it is a precise form of temperature control to stabilize workload temperature by adjusting the proportional, integral and

derivative parameters. It is also essential to fine-tune the PID parameters in order to achieve a stable and fast thermocycling temperature profiles.

Figure 6.10: Thermocycling profile with little PID control optimization (left) and with excessive PID control (right)

In Figure 6.10, we can observe two typical PCR thermal cycling profiles with little (left) and excessive (right) PID control on the system. Figure 6.10 (left) shows the thermal cycling profile before optimizing the PID parameters. Large overshoots and temperature fluctuations (about $\pm 5^{\circ}\text{C}$) around set temperatures can be observed. Figure 6.10 (right) shows a smooth temperature profile resulted by applying excessive PID control. This set of PID parameters results in a longer time to reach the set temperature. However, this is also the most stable temperature profile achievable. The maximum overshoots/undershoots are less than $\pm 0.2^{\circ}\text{C}$ from the set temperature.

Therefore, it is necessary to find an optimal temperature profile for the PCR thermal cycling that is capable of a rapid and sufficiently smooth and stable profile. The PID parameters are adjusted and fine-tuned until an optimized temperature profile with small overshoots/undershoots, little fluctuations and short durations is attained, as shown in Figure 6.11.

Figure 6.11: Thermocycling profile after PID optimization

Figure 6.11 shows a temperature profile with small overshoots/undershoots and fluctuations. The maximum overshoots/undershoots are less than $\pm 0.5^{\circ}\text{C}$ and fluctuations of less than $\pm 0.2^{\circ}\text{C}$ about the set temperature. A complete cycle takes about 36 seconds (to illustrate every steps of a whole PCR clearly, the duration of Denaturation is set as 10 seconds; the duration of Annealing is set as 10 seconds; the duration of Extension is set as 10 seconds.). As a result, a PCR experiment (with above settings) of 40 cycles can be completed in about 24 minutes.

6.3.4 Measured temperature profile of chip PCR system

To evaluate the simulation results of chip PCR system, a single PCR element coated with five RTD sensors is fabricated and used in this system. As shown in Figure 6.12, these sensors detect the strategical temperatures of a PCR element to investigate the heat transition and temperature uniformity during PCR process in the microchip. The thin film RTD sensors are coated in a $0.3 \times 0.3 \text{ mm}^2$ square area on the surfaces of cover or substrate; the thickness of them is $400 \text{ \AA}$; the resistance of them is 100Ω . Sensor 2 is coated at the center node of the cover bottom surface; sensor 3 is coated at the center node of the substrate top surface; sensor 4 is coated at the boundary node of the cover bottom surface; sensor 5 is coated at the boundary node of the substrate top surface, sensor 6 is coated at the center node of substrate bottom surface.

Figure 6.12: A cross-sectional view of a single PCR element. Node 1 is located at the centre of sample, the temperature at node 1 is assumed to be the mean of those of node 2 and node 3; node 2 is located at

the centre of sample top surface, the temperature at node 2 is detected by a thin film RTD sensor coated at the bottom surface of cover; node 3 is located at the centre of sample bottom surface, the temperature at node 3 is detected by a thin film RTD sensor coated at the top surface of substrate; node 4 is located at the boundary of sample top surface, the temperature at node 4 is detected by a thin film RTD sensor coated at the bottom surface of cover; node 5 is located at the boundary of sample bottom surface, the temperature at node 5 is detected by a thin film RTD sensor coated at the top surface of substrate; node 6 is located at the surface of heater, the temperature at node 6 is detected by a thin film RTD sensor coated at the surface of bottom layer.

Measured temperature variations of different nodes of the PCR element in stage 1 are illustrated as Figure 6.13. The thermal transition between 70 °C and 95 °C can be accomplished in 0.76 second. The ramping rate of node 1 is 32.86 °C/s. Obviously, the center nodes ramp faster than the boundary nodes, and the bottom nodes ramp faster than the top nodes.

Figure 6.13: Temperature variation at different nodes of PCR element in stage 1

Figure 6.14: Comparison of measured temperature and simulated temperature at node 1 in stage 1

The results from numerical simulation in Chapter 4 are compared with results from experimental validation, as shown in Figure 6.14. From the experimental data, the temperature increases at a slower rate due to the PID control. The simulated heating rate is $33.3\text{ }^{\circ}\text{C/s}$, while the measured heating rate is $32.86\text{ }^{\circ}\text{C/s}$. The difference between measured experiment and simulated condition is 1.34%.

Measured temperature variations of different nodes of the PCR element in stage 3 are illustrated as Figure 6.15. The thermal transition between 95°C and 60°C can be accomplished in 5.15 second. The cooling rate of node 1 is $6.79\text{ }^{\circ}\text{C/s}$. The temperature differences between these nodes are much less than those in heating processes. The maximum temperature difference among these nodes is $1.5\text{ }^{\circ}\text{C}$ in stage 3.

Figure 6.15: Temperature variation at different nodes of PCR element in stage 3

Figure 6.16: Comparison of measured temperature and simulated temperature at node 1 in stage 3

In Figure 6.16, we have a comparison of experimental data and numerical simulation during stage 3. Similarly, the measured temperature of node 1 shows a slower decrease than the simulation. This is also because of the PID control in cooling

process. The cooling rate difference between measured experiment and simulated condition is 15.4%.

Measured temperature variations of different nodes of the PCR element in stage 5 are illustrated as Figure 6.17. The thermal transition between 55 °C and 70 °C can be accomplished in 0.46 second. The ramping rate of node 1 is 32.82 °C/s. Similar with stage 1, the center nodes ramp faster than the boundary nodes; and the bottom nodes ramp faster than the top nodes.

Figure 6.18 shows a comparison of experimental data and numerical simulation in stage 5. The simulated heating rate is 33.3 °C/s, while the measured heating rate is 32.82 °C/s. The difference between measured experiment and simulated condition is 1.46%.

Figure 6.17: Temperature variation at different nodes of PCR element in stage 5

Figure 6.18: Comparison of measured temperature and simulated temperature at node 1 in stage 5

Based on above experimental data, the measured temperature profile of a complete PCR thermal cycle is illustrated in Figure 6.19, with the simulated temperature profile as a reference. Although the measured temperature transition is slightly slower than the simulated conditions, one complete PCR thermal cycle can still be accomplished in 8 seconds as shown in Figure 6.19. As the result, the rapid PCR can be realized using the microchip. The whole PCR process, which would normally costs 30 minutes, will be accomplished in about 4 minutes. It would improve the efficiency of clinical diagnostics dramatically.

Figure 6.19: Comparison of measured and simulated temperature profiles (represented by the temperature at node 1) of a complete PCR thermal cycle

6.3.5 Measured thermal cross-talk in PCR chamber array

To evaluate the simulation result of thermal cross-talk in PCR chamber array, a PCR chip with two adjacent elements is tested in chip PCR system. According to the Fourier's law of heat conduction, $\Phi = -\lambda A dT/dx$, where λ is thermal conductivity of the material (which is constant), A is the area (which is constant according to the chip design), dT/dx is the temperature distribution [91]; since heat transfer rate can not be detected directly; the temperature distribution of the PCR sample and common boundary is measured to demonstrate the heat transfer rate. As shown in Figure 6.20, thin film RTD sensors A, B, C, E, F and H are coated on the surfaces of the cover, the substrate and the common boundary to detect the temperatures in adjacent half PCR elements variation during a PCR thermal cycle. The temperature at node D is assumed as the mean of node C and E, while the temperature at node G is assumed as the mean of node F and H.

Figure 6.20: (a) Sketch of two adjacent PCR elements; (b) A cross-sectional view of two adjacent PCR elements. It is divide into the left half and the right half. Node A is located at the top surface of common boundary; Node B is located at the bottom surface of common boundary; Node C and F are located at the bottom surface of cover; Node E and H is located at the top surface of substrate. The temperatures of node A, B, C, E, F and H are detected by thin film Pt-RTD sensors. Node D and G is located at the center point of sample. The temperature at node D is assumed as the mean of node C and E, while the temperature at node G is assumed as the mean of node F and H.

To demonstrate the thermal cross-talk in the chamber array efficiently, the temperature profiles of the left half and right half (assuming the temperatures of node D and G to represent) PCR elements are controlled alternately. Assumed the signal from the temperature controller output to the solid state relay which connected with left heater is heating, the signal to the right half should be cooling. The temperature profiles of node A and B are illustrated in Figure 6.21, with the temperature profiles of controlled left (node D) and right (node G) half PCR elements as reference.

Figure 6.21: Comparison of measured and simulated per-cycle (8 sec) temperature profiles at different locations of PCR chamber array by effective alternate heating/cooling control (heat up by heat transfer from the heater, cool down by forced air convection in the convection channel with 4m/s air incoming velocity) with thermal cross-talk between adjacent PCR elements using active air flow insulation. The measured temperature profiles of the adjacent half PCR elements are similar with the simulated temperature profiles, thus the heat transfer rate should be similar.

As shown in Figure 6.21, the temperature distributions of two adjacent PCR elements are almost same with the simulated result in chapter 4 (the temperature difference maximum of node A, B, D and G between the measured condition and simulated condition is less than 3°C). Thus the heat transfer rate across adjacent PCR elements should be similar with the simulation. Because most heat transferred between the PCR elements is taken away by the air in convection channel (acting as the insulation material), almost no heat transfer via the common boundary from the high temperature PCR element to the low temperature PCR element during a PCR thermal cycle (8 second).

6.3.6 Measured effect of PCR element characteristics on PCR cycling speed

Different micro PCR chips with various characteristics (such as the thickness of substrate, the thickness of sample, and the thermal conductivity of substrate) of PCR element are fabricated and tested in the chip PCR system to investigate the effect of PCR element characteristics on PCR speed and temperature uniformity. Following

investigations are based on the temperatures of different nodes in the sample, as shown in Figure 6.22.

Figure 6.22: A cross-sectional view of PCR chamber. Node TC is located at the centre of sample top surface, the temperature at node TC is detected by a thin film Pt-RTD sensor coated on the bottom surface of cover; node BC is located at the centre of sample bottom surface, the temperature at node BC is detected by a thin film Pt-RTD sensor coated on the top surface of substrate; node C is located at the centre of sample, the temperature at node C is assumed as the mean of the temperatures of node TC and BC; node TB is located at the boundary of sample top surface, the temperature at node TB is detected by a thin film Pt-RTD sensor coated on the bottom surface of cover; node BB is located at the boundary of sample bottom surface, the temperature at node BB is detected by a thin film Pt-RTD sensor coated on the top surface of substrate.

6.3.6.1 Effect of PCR element geometry

The PCR elements with different thicknesses (0.1mm, 0.2mm and 1.1mm) of substrate are tested in chip PCR system.

(a) In heating period

Figure 6.23: Measured heating rate of the chip with various thickness of substrate

Figure 6.24: Comparison of measured and simulated heating rate of the chip with various thickness of substrate

As shown in Figure 6.23, the heating rate decrease when the thickness of the substrate increases. The maximum heating rate of node C (the center node of sample) is 32.86 °C/s. Because the sample thickness is 0.08mm, the heating rates are almost same across the thickness of the sample. The main difference of heating rate is located along the radius of the sample, but this difference is decreasing gradually when the

thickness of substrate increases. As shown in Figure 6.24, the measured heating rates are slightly lower than the simulated rates. The maximum of heating rate difference between them is 4.05%. With the increase of substrate thickness, the difference between the measured heating rate and simulated heating rate is approaching to 0.

(b) In cooling period

Figure 6.25: Measured cooling rate of the chip with various thickness of substrate

Figure 6.26: Comparison of measured and simulated cooling rate of the chip with various thickness of substrate

As shown in Figure 6.25, the cooling rate decrease when the thickness of substrate increase; the maximum cooling rate of node C (the center node of sample) is 6.79 °C/s. Due to the slow temperature transition rate, the cooling rate differences between different nodes in the sample are much less than those of heating rates. As shown in Figure 6.26, the measured cooling rates are obviously less than the simulated results. The maximum difference between them is 26%. It is caused by the PID control in the measured operation and a slight over-estimation of air flow velocity from the cooling fan.

The PCR chambers with different sample thicknesses (0.08mm, 0.2mm and 0.8mm) are also tested in the chip PCR system.

(a) In heating period

Figure 6.27: Measured heating rate of the chip with various thickness of sample

Figure 6.28: Comparison of measured and simulated heating rate of the chip with various thickness of sample

As shown in Figure 6.27, the heating rate decrease when the sample thickness increases. The maximum heating rate of node C (the center node of sample) is 32.86 °C/s. When the thickness of sample is less than 0.4mm, the main difference of heating rate is distributed along the radius of sample; but with the increase of thickness, the main difference of heating rate is distributed across the thickness. As shown in Figure 6.28, although the measured heating rates are slightly lower than the simulated rates, these two curves are almost same with the increase of thickness of sample.

(b) In cooling period

Figure 6.29: Measured cooling rate of the chip with various thickness of sample

Figure 6.30: Comparison of measured and simulated cooling rate of the chip with various thickness of sample

As shown in Figure 6.29, the cooling rate decrease when the thickness of sample increase; the maximum cooling rate of node C (the center node of sample) is 6.79 °C/s. As shown in Figure 6.30, the measured cooling rates are less than the simulated results. The maximum difference between them is 27.2%. It is also caused by the PID control in the measured operation and a slight over-estimation of air flow velocity from the cooling fan.

6.3.6.2 Effect of thermal conductivity of substrate

The PCR chambers with different thermal conductivity (polyimide 0.2W/mk, glass 1.1W/mk and Ni25w%Fe 13W/mk) of substrate are tested in the chip PCR system.

(a) In heating period

Figure 6.31: Measured heating rate of the chip with various thermal conductivity of substrate

Figure 6.32: Comparison of measured and simulated heating rate of the chip with various thermal conductivity of substrate

As shown in Figure 6.31, there is a peak (33 °C/s) at 1.4W/mk. The main difference of the heating rate is distributed along the radius of the sample. Actually another material, Silicon (the thermal conductivity is 150W/mk), is tested in the experiments. The heating rate of Silicon substrate can reach 35 °C/s, but the thermal cross-talk in PCR chamber array with the Silicon substrate is very obvious (discussed in next section, as shown in Figure 6.38). Thus Silicon not included, the x axis ranges from

0.2W/mk to 13W/mk. As shown in Figure 6.32, the measured heating rates are slightly lower than the simulated rates. But these two curves are almost same with the increase of thickness of substrate. The maximum difference between them is 6.25%.

(b) In cooling period

Figure 6.33: Measured cooling rate of the chip with various thermal conductivity of substrate

Figure 6.34: Comparison of measured and simulated cooling rate of the chip with various thermal conductivity of substrate

As shown in Figure 6.33, the cooling rate increases when the thermal conductivity of substrate also increases. The differences among the cooling rates of different nodes in the sample are less than 0.25 °C/s. As shown in Figure 6.34, the measured cooling rates are lower than the simulated results, but with the increase of thermal conductivity, the difference between them decreases gradually. The maximum difference between them is 11.25%.

6.3.7 Measured effect of PCR element characteristics on thermal cross-talk in PCR chamber array

Different PCR chamber array chips with various thickness of substrate, or thickness of sample, or thermal conductivity of substrate are fabricated and tested in the chip PCR system to investigate the effect of chamber characteristics on thermal cross-talk in PCR chamber array.

6.3.7.1 Effect of PCR element geometry

The PCR elements with different thicknesses (0.1mm, 0.2mm and 1.1mm) of substrate are tested in chip PCR system. The temperature investigated in this section is represented by the temperature of the sample center point (node 1 in Figure 6.12).

Figure 6.35: Measured heating rate of the chip with various thickness of substrate with/without thermal cross-talk

Figure 6.36: Measured cooling rate of the chip with various thickness of substrate with/without thermal cross-talk

As shown in Figure 6.35 and Figure 6.36, thermal cross-talk almost has no effect on the PCR chamber array in spite of thickness of substrate. The maximum difference between the heating rate curves with and without thermal cross-talk is 2.86%, while that of the cooling rate curves is 3.47%.

The PCR elements with different thicknesses (0.08mm, 0.2mm and 0.8mm) of sample are also tested in chip PCR system.

Figure 6.37: Measured heating rate of the chip with various thickness of sample with/without thermal cross-talk

Figure 6.38: Measured cooling rate of the chip with various thickness of sample with/without thermal cross-talk

As shown in Figure 6.37 and Figure 6.38, thermal cross-talk also has no effect on the PCR chamber array in spite of the thickness of sample. The maximum difference between the heating rate curves with and without thermal cross-talk is 4.8%, while that of the cooling rate curves is 2.74%.

6.3.7.2 Effect of thermal conductivity of substrate

The PCR elements with different thermal conductivity (polyimide 0.2W/mk, glass 1.1W/mk and Ni25w%Fe 13W/mk) of substrate are investigated in chip PCR system.

Silicon, which has extremely high thermal conductivity (150W/mk), is also tested as the substrate (Figure 6.39 (a)) in the chip PCR system. But as shown in Figure 6.39 (b), the cooling and heating processes between two adjacent elements were obviously affected by thermal cross-talk. The cooling process even can not be accomplished. Thus the material with high thermal conductivity, such as Silicon, can not be regarded as the suitable material for PCR chamber array.

Figure 6.39: (a) Sketch of two adjacent chambers with Silicon substrate; (b) Temperature profile in two adjacent PCR elements (chambers) with Silicon substrate (thickness of substrate is 0.1mm; thickness of sample is 0.08mm). The cooling process can not be accomplished due to the effect of thermal cross-talk.

Figure 6.40: Measured heating rate of the chip with various thermal conductivity of substrate with/without thermal cross-talk

Figure 6.41: Measured cooling rate of the chip with various thermal conductivity of substrate with/without thermal cross-talk

As shown in Figure 6.40, when the thermal conductivity of substrate is less than 1.5W/mk, the effect of thermal cross-talk can be ignored. But with the increase of thermal conductivity, the heating rate is gradually affected more and more by thermal cross talk between adjacent PCR elements. As shown in Figure 6.41, when the thermal conductivity of substrate is less than 2W/mk, the effect of thermal cross-talk can be ignored. Similar to the heating rate curves, with the increase of thermal conductivity, the cooling rate is also gradually affected by thermal cross talk between adjacent PCR elements. If the thermal conductivity is too large, the temperature of cooling chamber can not decrease after a short time of cooling process, just like the condition of silicon (Figure 6.39 (b)).

6.4 DNA melting analysis chip system

6.4.1 DNA melting analysis chip system setup

In addition to rapid temperature variation control in the on-chip PCR process, on-chip DNA melting analysis is another important process for the chip system. Precise

temperature control and temperature uniformity are the critical criterion. The DNA melting analysis chip system can be setup as shown in Figure 6.42.

Figure 6.42: Working principle of DNA melting analysis chip system

To investigate the temperature distribution within a chip, a set of RTD sensors are placed at different locations of microchip as shown in Figure 6.43. With these thin film RTD temperature sensors, the temperature control and temperature uniformity during DNA melting analysis process can be investigated.

Figure 6.43: A cross-sectional view of DNA melting analysis chip system. To reduce the reflection effect from the isolated chip container, it must be painted with black color. Node 1 is located at the centre of sample bottom surface, the temperature at node 1 is detected by a thin film RTD sensor coated at the top surface of substrate; node 2 is located at the centre of sample top surface, the temperature at node 2 is detected by a thin film RTD sensor coated at the bottom surface of cover; node 3 is located at the boundary of sample bottom surface, the temperature at node 3 is detected by the thin film RTD sensor coated at the top surface of substrate; node 4 is located at the boundary of sample top surface, the temperature at node 4 is detected by the thin film RTD sensor coated at the bottom surface of cover; node 5 is located at the center of the heater, the temperature at node 5 is detected by the thin film RTD sensor coated at the top surface of the bottom layer.

The DNA melting analysis chip system is a close loop control system, as shown in Figure 6.44. After calibration, the temperature signals from a thin film RTD sensor are input to a 24 bits A/D (Analog to Digital) device, and then collected by a microprocessor. The PID controller sends out digital commands to a 20 bits D/A device. The analog signals are amplified to control the microheater to achieve 0.01°C temperature control accuracy. During the experiment, the whole microchip was placed in a thermal insulation environment (thick plastic isolated chip container, as shown in Figure 6.43). Even though, a small change of boundary conditions may affect the temperature control precision. Thus, the surroundings around microchip should be sufficiently thermal-isolated or simultaneously thermal-controlled.

Figure 6.44: Principle of precise temperature control in chip system

6.4.2 Modules of DNA melting analysis chip system

This DNA melting analysis chip system comprises of the microfabricated heating elements, the high precision temperature control system, the optics system (described in Section 6.3.2) and the fluorescence detection/imaging system (DTA DISCOVERY

DS260E camera, described in Section 6.3.2). The photographs of this module are shown in Figure 6.45.

The principal component of this system is the high precision temperature control system. The temperature control accuracy is 0.01°C , and the temperature detection accuracy is 0.001°C . It is a customized device fabricated by Photonik Technologies Pte. Ltd. (Singapore). It employs an electronic design to reduce most of the noise from main power supply, shock noise, and white noise. Its key feature is the build-in Amplifier which uses extremely low bandwidth for band-pass filter and very low cut-off frequency for low-pass filter (0.1Hz).

Figure 6.45: (a) Photographs of DNA melting analysis system modules; (b) closed up view of the chip.

Figure 6.46: Design architecture of high precision temperature control system

The system block diagram is shown in Figure 6.46. The temperature sensor converts temperatures into electronic signal, and the signal is conditioned, adjusted and finally feed into a demodulator. Demodulated signal is transferred into precision 24-bit A/D converter then retrieved by microcontroller. The microcontroller processes this signal by the PID algorithm, and its output is sent to 20-bit D/A converter and conditioned to drive the heating components. The heating components heat up the samples thus temperature sensor gets a signal change. Thus, the close-loop feed back system is set up, and its final balance point is approached by PID control. [92]

6.4.3 Measured temperature profile of DNA melting analysis

To evaluate the simulation results of DNA melting analysis chip system, a PCR element coated with five RTD sensors is used in this system. As shown in Figure 6.43, these sensors detect the strategical temperatures of a PCR element to investigate heat transition and temperature uniformity during DNA melting analysis process in the microchip.

Figure 6.47: Measured temperature profile of a chamber during DNA melting analysis. The MTS represents the Melting Transition Step (defined in section 3.4.3). The ΔT represents the temperature difference among the nodes

Shown as Figure 6.47, when the duration of 0.01°C increments is set to 0.5sec, the maximum temperature difference in the sample is less than 0.001°C . Thus it is concluded that temperature uniformity during DNA melting analysis in the chip system can be guaranteed with the heating rate at 0.02°C/s .

6.4.4 Measured effect of PCR element characteristics on precise temperature control during DNA melting analysis

Different chips with various PCR element characteristics (such as the thickness of substrate, the thickness of sample, and the thermal conductivity of substrate) are fabricated and tested in the chip system to investigate the effect of chamber characteristics on the Melting Transition Time (defined in Section 3.4.3, shown as Figure 3.26) during DNA melting analysis.

6.4.4.1 Effect of PCR element geometry

The PCR elements with different thicknesses (0.1mm, 0.2mm, 0.5mm and 1.0mm) of substrate are tested in chip system. As shown in Figure 6.48, when the thickness of

substrate increases, the melting transition time also increases. Thermal delay is obvious (the Melting Transition Time is more than 1.0 sec) if the substrate is thicker than 0.15mm.

Figure 6.48: Comparison of measured and simulated melting transition time curve of the chip with various thickness of substrate

The PCR elements with different thicknesses (0.08mm, 0.2mm, and 0.8mm) of sample are detected in chip system. As shown in Figure 6.49, the thickness of sample increases at the same time with the melting transition time also increases. Similar with the effect of substrate thickness, thermal delay is obvious if the sample is thicker than 0.14mm.

Figure 6.49: Comparison of measured and simulated melting transition time curve of the chip with various thickness of sample

6.4.4.2 Thermal conductivity of substrate

The chambers with different thermal conductivity (polyimide 0.2W/mk, glass 1.1W/mk and Ni25w%Fe 13W/mk) of substrate are detected in chip system. As shown in Figure 6.50, the thickness of sample increases, while the melting transition time decreases. Thermal delay is obvious if thermal conductivity of substrate is less than 0.46W/mk.

Figure 6.50: Comparison of measured and simulated melting transition time curve of the chip with various thermal conductivity of substrate

6.5 PCR experiments and results on chip PCR system

The ultimate aim for the development of the PCR chip is to perform PCR successfully. The recipe for the PCR mix as well as the important parameters of PCR experiments, such as specific dwelling temperatures for each phase of PCR and their dwelling time, will be presented in this section. Lastly, results from real-time DNA amplification detection will be shown.

6.5.1 PCR mixture preparation

The PCR mixture is prepared for 2 chambers in chip PCR system and 2 tubes in Rotor gene PCR machine (developed by Corbett Research). Sample volume per chamber/tube is 1 μ l. The second column in Table 6.3 describes the composition of the PCR mix that is sufficient for sample volume of one chamber. The third column shows that total PCR mix volume for 2 chambers (1 μ l/chamber) tested in chip PCR system and 2 tubes (1 μ l/tube) tested in Rotor gene PCR machine to be prepared. The extra capillary volumes are prepared to accommodate the wastage during sample loading.

Table 6.3: Recipe for PCR mixture

Mixture Component	Per-reaction	5 reactions	Final Concentration
Forward Primer (10 μ M)	0.03 μ l	0.15 μ l	0.3 μ M
Reverse Primer (10 μ M)	0.03 μ l	0.15 μ l	0.3 μ M
dNTPs (10 mM)	0.1 μ l	0.5 μ l	0.2 mM
BSA (10 mg/ml)	0.1 μ l	0.5 μ l	1.0 μ g/ μ l
Buffer (10X)	0.1 μ l	0.5 μ l	1X
MgCl ₂ (50 mM)	0.06 μ l	0.3 μ l	3.0 mM
SYBR Green (100X)	0.1 μ l	0.5 μ l	1X
MQ Water	0.37 μ l	1.85 μ l	
Pt Taq DNA polymerase (5U/ μ l)	0.01 μ l	0.05 μ l	0.05 U/ μ l
Volume	0.9 μ l	4.5 μ l	
DNA Template	0.1 μ l	0.5 μ l	
Total Volume	1 μ l	5 μ l	

Primer-pairs for PCR

The PCR primer pair was designed to amplify a 77 base-pair of BNI-1 fragment of SARS and to flank the region of amplification. The primers are designed to avoid

sequences, which would produce internal secondary structures. Ideally, both primers should anneal at the same temperature.

The sequence of the forward and reverse primers are 5'-TTATCACCCGCGAAG AAGCT-3' and 5'-CTCTAGTTGCATGACAGCCCTC-3' respectively [93]. Regardless of primer choice, the final concentration of the primer in the reaction mix is at a final concentration of 0.3 μ M in a 1 μ l reaction.

dNTPs

PCR Nucleotide Mix (Promega Cat. # C1141, Promega corporation, US) is a premixed solution containing the sodium salts of dATP, dCTP, dGTP and dTTP, each at a concentration of 2mM in water. This solution is ready to use and is optimized for standard polymerase chain reactions and specialty approaches including hot-start and reverse transcription PCR (RT-PCR). The final concentration of the dNTPs in the reaction mix was redundant of 0.2mM in a 1 μ l PCR reaction.

Bovine Serum Albumin (BSA)

Acetylated BSA (Promega Cat. #R3961, Promega corporation, US) can be used as an enzyme stabilizer or as a carrier protein. It is dialyzed extensively with deionized water to remove impurities.

Taq DNA Polymerase PCR Buffer

The Taq DNA Polymerase PCR Buffer (Invitrogen Cat. No. 10966-018, Invitrogen Corporation, Carlsbad, California, US) is to be used with Taq DNA Polymerase only. It is supplied as a 10X concentrate and is to be diluted 1:10 (1 part buffer + 9 parts other components = 10 parts final reaction volume).

Buffer Composition (10X): 200 mM Tris-HCl (pH 8.4), 500 mM KCl.

Magnesium Chloride (MgCl₂)

Magnesium concentration is a crucial factor affecting the performance of Taq DNA polymerase. Reaction components, including template DNA, chelating agents present in the sample (e.g., EDTA or citrate), dNTPs and proteins, can affect the amount of free magnesium. In the absence of adequate free magnesium, Taq DNA polymerase is inactive. Conversely, excess free magnesium reduces enzyme fidelity and may increase the level of nonspecific amplification. Magnesium ion stabilized DNA duplex, hence high concentration of MgCl_2 decreases the efficiency of PCR. For these reasons, it is important to empirically determine the optimal MgCl_2 concentration for each reaction. In this case, the final concentration of the MgCl_2 (Invitrogen Cat. No. 10966-018, Invitrogen Corporation, Carlsbad, California, US) in the reaction mix is at a final concentration of 3mM in a 1 μ l reaction.

Fluorescent Dye

SYBR Green (Cambrex Part No. 50513, Cambrex Corporation, US) is a highly sensitive fluorescent stain for detecting dsDNA and oligonucleotides. SYBR Green I fluorescent signal is greatly enhanced when the dye is bound to nucleic acids. It is ideal for detecting small amounts of DNA in low copy/target number PCR products, apoptosis studies, and heteroduplex analysis.

MQ Water

MQ water can be self-prepared by autoclaving deionized water at about 121°C. It is highly purified water that is low in ions, particles, organic matter and colloids, which could affect the PCR reaction adversely.

Platinum® Taq DNA Polymerase

Platinum® Taq DNA Polymerase (Invitrogen Cat. No. 10966-018, Invitrogen Corporation, Carlsbad, California, US) is recombinant Taq DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the denaturation step in PCR cycling at 94°C,

thereby providing an automatic hot start for Taq DNA polymerase in PCR. Hot starts in PCR provide increased sensitivity, specificity, and yield, while allowing assembly of reactions at room temperature. The use of this antibody helps reduce PCR optimization requirements, reaction set-up time and effort, handling of reaction components, and contamination risk, thereby improving PCR results. Platinum® Taq DNA Polymerase is supplied at a concentration of 5 units per µl.

Cloning of SARS DNA fragment

SARS RNA was quantified using the Ribogreen kit from Molecular Probes, Inc. (Eugene, OR). Reverse Transcription was performed using Invitrogen's (Carlsbad, CA) Superscript First-Strand Synthesis System with random primers according to the manufacturer's instruction. After reverse transcription, the enzyme was heat inactivated and then the sample was treated with RNase H. PCR was performed separately using Pfu DNA Polymerase from Promega (Madison, WI) and the SARS cDNA. Forward primer 5'-ggggtaccatacctcagcggtgatataaagttcaag-3' and reverse primer 5'-agaattcgaagcctttatacatgagtggtataagatg-3' was used to amplify a region between nucleotide position 18031 and 18424 of the SARS coronavirus CUHK-AGO1 genome. This region encompassed the BNI-1 fragment described by Drosten et. al. [94]. Additional nucleotide sequence on the primers was added to introduce Kpn1 and EcoRI restriction site respectively. PCR condition on a MJ Research PTC100 thermocycler is : initial 95°C – 1min, 35 cycles of 95°C – 45sec, 55°C – 1min and 72°C – 1min, ending with 72°C – 10min and 4°C – 30min. After PCR, agarose gel electrophoresis was performed and a major band of approximately 400 base pairs was excised. This amplified DNA fragment, was extracted from the gel using Qiagen (Hilden, Germany) Qiaquick gel extraction kit and then double digested with Kpn1 and EcoRI. The digested DNA fragment was ligated using T4 DNA ligase into double digested pGEM-3Z vector from Promega, and transformed into XL-1B.

DNA Template

400 base-pair fragment of SARS RNA was RT-PCR amplified and cloned into pGEM-3Z vector. The 77 base-pair fragment (TTATCACCCCGCGAAGAAGCTATTCGTCACGTTCGTGCGTGGATTGGCTTTGATGTAGAGGGCTGTCATGCAAC TAGAGA) was cloned between Kpn I and Eco R I restriction sites [95]. The PCR reaction mixture contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 0.2 mM each dATP, dCTP, dTTP and dGTP, 3 mM MgCl₂, 0.3 μ M each forward and reverse primer, 1 μ g/ μ l of BSA, 0.1 U/ μ l of Platinum Taq DNA polymerase (Invitrogen), 1X SYBR Green I (Cambrex Biosciences Rockland, Inc.) and 0.1 ng/ μ l of SARS DNA cloned in pGEM-3Z vector. [96]

6.5.2 Sample loading and sealing of PCR chambers

1 μ l sample is loaded into each PCR element (in chip PCR system) or each tube (in Rotor gene PCR machine) by pipette manually. In Rotor gene PCR machine, the tube is simply sealed by a cover connected with the tube. In the chip PCR system, after the PCR mixture is loaded successfully into the chamber array, the sample has to be effectively sealed to prevent contamination and evaporation during PCR thermal cycling. The silicone elastomer is a transparent and viscous solution. Due to its viscosity, it cannot be mixed with PCR sample. The SYLGARD 184 silicone elastomer requires about 20 minutes at 70°C to be fully cured. The following process is conducted to seal the sample inside the chamber.

At the first step, the PDMS elastomer is still a liquid mixture. The top layer is placed upside down, and then the PDMS liquid is loaded on the surface of the top layer. The thickness of the PDMS layer is less than 60 μ m (half thickness of the cover).

At the second step, place the top layer with PDMS on it into an oven at 70°C for 15 minutes to enable the PDMS elastomer to be sufficiently cured for handling.

At the third step, load the PCR sample into PCR elements.

At the fourth step, take the top layer with PDMS out of oven and reverse its sides. The PDMS would stick on the surface of the top layer and could not form a liquid droplet.

At the fifth step, place the top layer on the top of the middle layer and apply pressure on them. Thus the PCR sample is sealed into the PCR element.

At the sixth step, replace the whole structure with PCR sample into the oven at 70°C for 5 minutes to enable the PDMS to be fully solidified to effectively encapsulate the PCR elements to prevent sample evaporation and contamination.

At the seventh step, take the PCR chip outside the oven, and the PCR sample is successfully sealed into the chambers (shown as Figure 6.51).

Figure 6.51: A cross-sectional view of PCR chip after sealing. The chamber structure is labelled with grey color; the sample (the volume is 1 μ l) is labelled with yellow color; the PDMS is labelled with blue color.

After loading and sealing, PCR experiments can be carried out in chip PCR system and Rotor gene PCR machine.

6.5.3 PCR experiment results

6.5.3.1 Chip PCR system performance experimental result

The PCR mix can be prepared based on the recipe presented in section 6.5.1. The PCR samples are loaded and sealed on the chip as seen in Figure 6.52. For evaluation with the commercial PCR machine, two tubes contain same solution (one positive control, one negative control) carry on PCR experiments in Rotor gene PCR machine

(developed by Corbett Research). Table 6.4 shows the parameters of the PCR experiment that is carried out. Figure 6.53 shows the temperature profile in chip PCR system with the PCR thermal cycling conditions shown in Table 6.4.

Figure 6.52: Plan view of PCR chip of 4 PCR elements. Element 1 and 4 have not loaded PCR mix, while element 2 is loaded with PCR mix of positive control (10pg/ μ l SARS DNA template, 2.9×10^7 DNA copy number). Element 3 is loaded with PCR mix of negative control (without DNA template). Element 2 and 3 are controlled by same PCR temperature and time parameters as Table 6.4.

Table 6.4: PCR thermal cycling conditions

S/No	Stage	Temperature ($^{\circ}$ C)	Time (sec)
1	Initialization	95	120
2	Denaturation	95	1
3	Annealing	58	1
4	Extension	72	1
5	Final Extension	72	600
	Number of cycles	40

Figure 6.53: Measured temperature profile of PCR thermal cycling in chip PCR system. (a) 20 cycles; (b) one cycle.

The results from real-time fluorescence detection during PCR experiment on chip PCR system are collected and presented below in Figure 6.54.

Figure 6.54: Measured fluorescence intensity vs. cycle number obtained from chip PCR system. The green curve represents the fluorescence intensity of the positive control sample (10pg/ μ l SARS DNA template, 2.9×10^7 DNA copy number). The red curve represents the fluorescence intensity of the negative control sample (without DNA template). An obvious fluorescence increase is observed at the 18th cycle. The PCR sample is amplified successfully.

As shown in Figure 6.54, an obvious increase in fluorescence intensity at the 18th cycle was detected in the positive control PCR mix. Thus the DNA templates were amplified successfully during this PCR experiment. Although there should be no fluorescence increase in the negative control PCR mix, a slight increase of fluorescence intensity can also be detected under negative control. That is because some primer-dimer would be synthesized approaching the end of this experiment [97]. The slight fluctuations observed in the profile are likely due to the presence of electrical noise during signal measurement and transmission.

A same batch of PCR mix was used in the Rotor gene PCR machine developed by Corbett Research to compare the performance of chip PCR system. If the PCR experimental parameters were set as same as those in Table 6.4, Rotor gene PCR machine can not achieve the proper PCR products with 1 second incubation time for PCR steps (denaturation, annealing and extension), as shown in Figure 6.55.

Figure 6.55: Plotted PCR (1 second incubation time) results of fluorescence intensity vs. cycle number obtained from Rotor gene PCR machine. The green curve represents the fluorescence intensity of the positive control sample (10pg/ μ l SARS DNA template, 2.9×10^7 DNA copy number). The black line represents the fluorescence intensity of the negative control sample (without DNA template). The fluorescence intensity has not the proper relationship with PCR cycle number. The PCR sample has not been amplified properly.

Figure 6.56: Plotted PCR (30 second incubation time) results of fluorescence intensity vs. cycle number obtained from Rotor gene PCR machine. The green curve represents the fluorescence intensity of the positive control sample (10pg/ μ l SARS DNA template, 2.9×10^7 DNA copy number). The black line represents the fluorescence intensity of the negative control sample (without DNA template). An obvious fluorescence increase is observed at the 18th cycle. The PCR sample is amplified successfully.

As shown in Figure 6.55, the PCR process with 1 second incubation time cannot detect the fluorescence increase in Rotor gene PCR machine. The fluorescence intensity of the PCR sample remains low and has no relationship with the cycle number. The PCR sample has not been amplified properly. Thus all the incubation time for PCR steps were changed from 1 second to 30 seconds. Result from it is shown in Figure 6.56.

As shown in Figure 6.56, compared with the results from chip PCR system, a similar fluorescence intensity profile was achieved in Rotor gene PCR machine. Figure 6.56 also shows an increase in fluorescence at the 18th cycle. That indicates that the chip PCR system has successfully amplified the DNA sequence at the specific targeted region, as well as Rotor gene PCR machine.

The results from chip PCR system and Rotor gene PCR machine are checked by running the amplified PCR products in agarose gel electrophoresis. A band representing the amplified DNA is visible around 100 base pairs (the SARS DNA template is 77 base pairs.) after ethidium bromide staining, as shown in Figure 6.57. A photograph of the results from the agarose gel taken after electrophoresis is shown below in Figure 6.57. The agarose gel contained 0.5µg/ml Ethidium Bromide (161-0433, BioRAD). 0.1µg/µl DNA ladder (N3231S NEB BioLabs) and 6X Loading Dye (G190A Promega) were used for DNA agarose gel electrophoresis.

Figure 6.57: Photograph of DNA electrophoresis on agarose gel. The PCR sample volume is 1µl. Lane M is 100 base-pair DNA ladder (NEB BioLabs, 500µg/ml, diluted with water to 0.1µg/µl, 6X dye). Lane 1 is PCR products of element 3 (negative control) from chip PCR system. Lane 2 is PCR products

of element 2 (positive control) from chip PCR system. Lane 3 is PCR products from Rotor gene PCR machine (negative control). Lane 4 is PCR products from Rotor gene PCR machine (positive control).

Hence, as shown in Figure 6.57, bands are visible in Lanes 2 and 4, as they are PCR products with SARS DNA template added. Because of the small volume of PCR sample, the PCR products (visible bands) are fader than the DNA ladder. As shown in Figure 6.57, the products from chip PCR system and Rotor gene PCR machine are same after electrophoresis on agarose gel. From these results, it shows that the recipe of PCR mix is prepared properly and the chip is capable of DNA amplification by PCR. Thus, it can be concluded that the performance of chip PCR system is better than that of Rotor gene PCR machine (using less time to obtain the same results). Rapid PCR process is achieved in chip PCR system.

In addition, to evaluate the chip sensitivity to DNA template concentration, six recipes for the PCR mix, each having different DNA copy number, are prepared. PCR tests are carried out with the chip PCR system (the six samples with different concentration and one negative control sample are loaded onto two PCR chips and controlled under same temperature profile at same time) and their results are plotted in Figure 6.58. As shown in Figure 6.58, PCR mix of lower DNA copy number exhibits a later fluorescence increase. The green curve represents the PCR sample with 10pg/ μ l DNA template final concentration; the deep blue curve represents the PCR sample with 1pg/ μ l DNA template final concentration; the yellow curve represents the PCR sample with 100fg/ μ l DNA template final concentration; the violet curve represents the PCR sample with 10fg/ μ l DNA template final concentration; the sky blue curve represents the PCR sample with 1fg/ μ l DNA template final concentration; the pink curve represents the PCR sample with 100ag/ μ l DNA template final concentration; It can be seen a fluorescence increase from PCR mix of 10pg/ μ l DNA template final concentration (2.9×10^7 DNA copy number) at around 17.7th cycle, whereas the PCR mix of decrease of 10-fold DNA copy number separately at around 19.9th, 23.7th, 26.6th, 29.4th, 31.7th cycle. That is because lower starting DNA copy number is present to intercalate the dye, which results in a later amplification. Hence, the chip PCR

system is sensitive to DNA template concentration (DNA copy number) and PCR are performed successfully.

Figure 6.58: Measured fluorescence results and standard curve of SARS DNA with different concentration. (a) Amplification of 10-fold serially diluted SARS DNA template from 10pg/ul (2.9×10^7 copy number) to 100ag/ul (29 copy number) (positive control). The red curve represents the PCR sample without DNA template (negative control); the threshold is set to the level at 10 times of the fluorescence noise of the chip system. (b) The standard curve of 10-fold serially diluted SARS DNA template.

6.5.3.2 PCR experimental result with thermal cross-talk control

In this experiment, The PCR mix and PCR experimental parameters are same with above experiment in chip PCR system, except the thermal cross-talk control. The PCR samples are loaded on the chip as shown in Figure 6.59.

Figure 6.59: Plan view of PCR chip of 4 PCR elements. Element 2 and 3 are loaded with PCR mix of positive control (10pg/ μ l SARS DNA template, 2.9×10^7 DNA copy number). Element 1 and 4 are loaded with PCR mix of negative control (without DNA template)

Figure 6.60: PCR temperature profiles of chip PCR system under thermal cross-talk control by active air insulation. The red curve represents the temperature transition of element 1 and 2; the blue curve represents the temperature transition of element 3 and 4. (a) 20 cycles; (b) one cycle.

Element 1 and 2 are alternately heated/cooled when element 3 and 4 are cooled/heated as Figure 6.60. With this control, the effect of thermal cross-talk in chip PCR system is investigated and evaluated. The results from real-time fluorescence detection during PCR process on chip PCR system with thermal cross-talk control are collected and presented in Figure 6.61.

Figure 6.61: Measured fluorescence intensity vs. cycle number obtained from chip PCR system with thermal cross-talk control. The green curve represents the fluorescence intensity of the sample under positive control in PCR element 2 (10pg/ μ l SARS DNA template, 2.9×10^7 DNA copy number). The red curve represents the fluorescence intensity of the sample under positive control in PCR element 3 (10pg/ μ l SARS DNA template, 2.9×10^7 DNA copy number). The blue curve represents the fluorescence intensity of the sample under negative control in PCR element 1. The yellow curve represents the fluorescence intensity of the sample under negative control in PCR element 4. The PCR samples in PCR element 2 and 3 are amplified properly (the fluorescence of them both increase at the 18th cycle) under thermal cross-talk control. The thermal cross-talk cannot affect the PCR results.

As shown in Figure 6.61, there is no effect of thermal cross-talk between adjacent PCR elements on the performance of PCR amplification. The fluorescence intensities of PCR sample in the PCR element 2 and 3 both increase at the same cycle (18th

cycle). The slight differences between the fluorescence intensity curves of PCR element 2 and 3/ 1 and 4 can be regarded as the fluctuations caused by noise. Thus it is confirmed that no thermal cross-talk is occurred in PCR chamber array. Every PCR element in the chip PCR system can be controlled by different temperature profiles independently and achieves the proper PCR products respectively as well.

6.6 DNA melting curve analysis experiments and results on chip system

After PCR process, the PCR products should be analyzed to check whether the target fragment of DNA was amplified. But the electrophoresis gel and current commercial PCR machine (for example, Rotor gene machine, in which the detection accuracy is $\pm 0.1^{\circ}\text{C}$, the control accuracy is 1°C and the ramping rate for DNA melting process is $5^{\circ}\text{C}/\text{min}$ [103]) can not clearly and precisely detect two similar PCR products with only one or two base-pair difference.

With the high precision temperature controller (the control accuracy is 0.01°C), high resolution detection optic system, and minimized chip including RTD sensor (the detection accuracy is 0.001°C) and micro heater, precise temperature scan (the ramping rate for DNA melting process is $1.2^{\circ}\text{C}/\text{min}$) and fluorescence detection can be realized in chip system.

Before detecting the real DNA templates which are different by one or two base pair, the two close melting temperatures would be simulated by different concentrations of salt to achieve the general information of the detectable resolution during DNA melting analysis. The melting temperature (T_m) of the amplicon defined by a primer pair is computed from the standard equation in Bolton and McCarthy (PNAS 84:1390 1962) as presented in Sambrook, Fritsch and Maniatis, Molecular Cloning [96,97]:

$$T_m = 81.5 + 16.6(\log_{10} ([\text{Na}^+])) + 0.41(\%GC) - 600/\text{length} \quad (6.1)$$

where $[\text{Na}^+]$ is the molar sodium concentration, %GC is the percent of Gs and Cs in the sequence, and length is the length of the sequence. The assumption for this formula is that the sequence of the DNA template must be not symmetric. The DNA

template must contain at least one G/C, and the length of it must be longer than 8 base pairs.

Assuming the lengths of the DNA templates are same, the melting temperature difference has direct proportion with the variation of the percent of Gs and Cs in the sequence, as shown in Figure 6.62. Assuming that only one base pair is different in the sequence of DNA template, the melting temperature difference is inversely proportional with the length of DNA, as shown in Figure 6.63.

Figure 6.62: Melting temperature difference of the DNA templates with same length and variable percent of Gs and Cs. ΔT_m represents the DNA melting temperature difference; $\Delta \%GC$ represents the difference of the percent of Gs and Cs in the sequences of DNA templates. The suitable detectable range of $\Delta \%GC$ in the DNA melting chip system is from 0.0244 to 4.87.

Figure 6.63: (a) Melting temperature difference of the DNA templates with variable length (0 ~ 500 base pair) and only one base pair difference in the sequence. (b) Melting temperature difference of the DNA templates with variable length (0 ~ 4000 base pair) and only one base pair difference in the sequence. ΔT_m represents the DNA melting temperature difference. The suitable detectable range of DNA length in the DNA melting chip system is from the shortest length (10 base pair) to 4100 base pair.

As shown in Figure 6.62, when the percent of Gs and Cs in the DNA template sequence is higher, it is easier to detect the difference of melting temperatures. Most

commercial machines can tell 2°C difference definitely, but none of them could distinguish such small difference as 0.01°C. The chip system can perform precise temperature control, even 0.01°C, so the suitable range of the GC percentage difference for the chip system is from 0.025 to 4.87.

As shown in Figure 6.63, when the length of the DNA template is longer, the difference of the melting temperatures is smaller. Because the chip system can detect 0.01°C, the suitable range of the DNA template is below 4000 base pairs.

For example of 77 base-pair SARS DNA template (described in Section 6.5.1), the difference of melting temperature caused by the one base-pair difference of the sequence (assumption: only percentage of Gs and Cs is changed) is shown as below:

$$\Delta T_m = 0.41(\Delta\%GC) = 0.41 \times [x - (x-1)] \times 100/77 = 0.41 \times 100/77 = 0.532^\circ\text{C}$$

To simulate the 0.532°C temperature difference, the concentration of salt would be changed as below:

$$\log_{10} [\text{Na}^+]/[\text{Na}^+]' = 0.532/16.6 = 0.032 ,$$

$$\text{Thus, } [\text{Na}^+]/[\text{Na}^+]' = 1.076$$

Hence, it is concluded that PCR products with 1 unit and 1.076 unit NaCl concentrations can simulate the difference of one base pair in sequence.

After the PCR process, 0.01mM NaCl and 0.01076mM NaCl are respectively added to the PCR products achieved from above on-chip experiments, while the total volume of the mixtures is 1μl. As a comparing reference, DNA melting analysis is carried out in Rotor gene machine. With the x axis set to temperature (T), the fluorescence intensity detected (F) and the first-derivative fluorescence intensity (-dF/dT) are illustrated in Figure 6.64 and 6.65.

Figure 6.64: Plotted results of fluorescence intensity vs. temperature obtained from Rotor gene machine during DNA melting curve analysis. The fluorescence intensity decreases smoothly.

Figure 6.65: Plotted results of $-dF/dT$ vs. temperature obtained from Rotor gene machine during DNA melting curve analysis. The $-dF/dT$ curve is smooth. The melting peak width, defined as the width at the 1/2 peak height, of the PCR product with 0.1mM NaCl is 5.15°C; the melting peak width of the PCR product with 0.1067mM NaCl is 6.86°C. The sky blue curve represents negative control (water).

As shown in Figure 6.64, the decrease of the fluorescence intensity is smooth during DNA melting process. The widths of the DNA melting peak are more than 5°C (as shown in Figure 6.65). The melting temperature of the PCR product can not be detected accurately, and the value of the melting temperature only could be calculated by the software.

The same problems exist in most commercial machine for DNA melting curve analysis. LightTyper is a recent (April, 2004) product from Roche Company. The heating rate can reach as small as 0.05°C/sec; the thermal uniformity is ± 0.4 across the plate; and the single-base probe resolution is single base changes by T_m shifts of 2°C or greater. [104] The raw melting curve data tracks are shown in Figure 6.66; the melting peak curves are shown in Figure 6.67.

Figure 6.66: Plotted raw melting curve data tracks obtained from LightTyper machine during DNA melting curve analysis. The fluorescence intensity decreases smoothly.

As shown in Figure 6.66, the decrease of the fluorescence intensity is also smooth during DNA melting process, which is similar with Rotor gene machine. The widths of the DNA melting peak are normally more than 4°C (as shown in Figure 6.67). Although the LightTyper can analyze the shapes of up to 384 melting curves and groups curves with similar shapes in a single run, it can not give the melting temperature values very accurately.

Figure 6.67: Plotted melting peak curves obtained from LightTyper machine during DNA melting curve analysis. The melting peak widths of the PCR products are mostly wider than 4°C.

But with the control temperature profile achieved in chip system shown in Figure 6.47, the DNA melting curve analysis is conducted on chip using the same PCR products with the experiment conducted in Rotor gene machine. The fluorescence intensity detected from the chip system are collected and presented as Figure 6.68. After the first-derivative analysis, the DNA melting peaks would be obvious shown as Figure 6.69.

Figure 6.68: Plotted results of fluorescence intensity vs. temperature obtained from DNA melting chip system during DNA melting curve analysis. The fluorescence intensities of the PCR products decrease sharply at the melting temperatures.

Figure 6.69: Plotted results of $-dF/dT$ vs. temperature obtained from chip system during DNA melting curve analysis. The peak width of the PCR product with 0.1mM NaCl is 0.072°C ; the peak width of the PCR product with 0.1076mM NaCl is 0.095°C . The melting temperature of the PCR product with 0.1mM NaCl is 83.042°C ; the melting temperature of the PCR product with 0.1076mM NaCl is 83.533°C . The blue curve represents negative control (water).

As shown in Figure 6.68, the fluorescence intensity of the PCR product decreases sharply at the melting temperature. Thus in Figure 6.69, the width of the melting peak is very narrow (less than 0.1°C). The accurate value of melting temperature can almost be read from Figure 6.69. According to the data analysis, the melting peak of PCR product with 0.1mM NaCl is located at 83.042°C , while the melting peak of PCR product with 0.1076mM NaCl is located at 83.533°C . The difference of melting temperature between them is 0.491°C . It is very close to the theoretical value (0.532°C) obtained from the formula. Thus the PCR products (For example, the 77 base-pair SARS DNA PCR product) with only one base pair difference can be successfully detected using chip system.

From above results, only one base pair difference in a 77 base-pair PCR product could be detected obviously. But for the detection accuracy, even one base pair difference in a 4000 base-pair PCR product would be detected, which satisfied the normal requirements (the length of normal PCR product is less than 400 base-pair) perfectly.

Two real 189 base-pair PCR products (ATAGGACAAGGGGTGGAAGGGCAGGA GCTCACACCGCACCGGGCTGGCCTGACACAATGGTAGCTCTGAAGGGAGG GGAAGAGAGAGGCCTGGGT[G/A]TGACCTGACACCTGCCGCTGCTTGAGT GGACAGCAGCTGGACTGGGTGGGCCCCACAGTGGTCAGCGATTCTGCCA AGTAGGGTTTAGCCGG; the forward primer is 5'-ATAGGACAAGGGGTGGAA GG-3'; the reverse primer is 5'-CCGGCTAAACCCTACTTGGC-3'.) with only one base pair difference [G/A] in the sequence are conducted for the DNA melting analysis in the chip system. According to formula (6.1), for the 189 base-pair PCR products, the difference of melting temperature caused by the one base-pair difference of the sequence (A at the 97th position is changed to G) is shown as below:

$$\Delta T_m = 0.41(\Delta\%GC) = 0.41 \times [x - (x-1)] \times 100/189 = 0.41 \times 100/189 = 0.217^\circ\text{C}$$

The fluorescence intensity detected from the Lightcycler machine (Roche, the detail specifications of Lightcycler are shown in Section 2.8) are collected and presented as Figure 6.70. After the first-derivative analysis, the DNA melting peaks of the real PCR products with one base pair difference are shown as Figure 6.71.

Figure 6.70: Plotted results of fluorescence intensity vs. temperature obtained from the real PCR product with one base pair difference (A/G) in Lightcycler machine during DNA melting curve analysis. The black curve represents the PCR product with A at 97th position; the red curve represents the PCR product with G at 97th position. The fluorescence intensities of the PCR products decrease smoothly at the melting temperatures.

Figure 6.71: Plotted results of $-dF/dT$ vs. temperature obtained from the real PCR product with one base pair difference (A/G) in Lightcycler during DNA melting curve analysis. The peak width of the PCR product with A at 97th position is 4.9°C; the peak width of the PCR product with G at 97th position is 5.1°C. The blue curve represents negative control (water).

As shown in Figure 6.70, the fluorescence intensity of the PCR product decreases smoothly at the melting temperature. The widths of the DNA melting peak are about 5°C (as shown in Figure 6.71). The Lightcycler can only discriminate melting temperature shifts of 2°C or greater [40, 41]. The melting temperature of the PCR product can not be detected accurately. Thus it is not suitable to use Lightcycler for precise DNA melting analysis.

The DNA melting analysis of the same PCR products are conducted in Rotor gene machine. The fluorescence intensity detected from Rotor gene machine are collected and presented as Figure 6.72. The DNA melting peaks of the real PCR products are shown as Figure 6.73. As shown in Figure 6.72, the fluorescence intensity of the PCR product collected from Rotor gene machine decreases more smoothly than that of Lightcycler at the melting temperature. As shown in Figure 6.73, the widths of the DNA melting peak are also larger than that of Lightcycler. The melting temperature of the PCR product can not be detected accurately in Rotor gene machine.

Figure 6.72: Plotted results of fluorescence intensity vs. temperature obtained from the real PCR product with one base pair difference (A/G) in Rotor gene machine during DNA melting curve analysis. The black curve represents the PCR product with A at 97th position; the red curve represents the PCR product with G at 97th position. The fluorescence intensities of the PCR products decrease smoothly at the melting temperatures.

Figure 6.73: Plotted results of $-dF/dT$ vs. temperature obtained from the real PCR product with one base pair difference (A/G) in Rotor gene during DNA melting curve analysis. The peak width of the PCR product with A at 97th position is 8.8°C; the peak width of the PCR product with G at 97th position is 10.1°C. The blue curve represents negative control (water).

With the reference of Lightcycler and Rotor gene machine, the DNA melting analysis of same PCR products is conducted in the DNA melting chip system. The fluorescence intensity detected from the chip system are collected and presented as

Figure 6.74. After the first-derivative analysis, the DNA melting peaks of the real PCR products with one base pair difference would be obvious shown as Figure 6.75.

Figure 6.74: Plotted results of fluorescence intensity vs. temperature obtained from the real PCR product with one base pair difference (A/G) in chip system during DNA melting curve analysis. The black curve represents the PCR product with A at 97th position; the red curve represents the PCR product with G at 97th position. The fluorescence intensities of the PCR products decrease sharply at the melting temperatures.

Figure 6.75: Plotted results of $-dF/dT$ vs. temperature obtained from the real PCR product with one base pair difference (A/G) in chip system during DNA melting curve analysis. The peak width of the PCR product with A at 97th position is 0.097°C; the peak width of the PCR product with G at 97th position is 0.071°C. The melting temperature of the PCR product with A at 97th position is 66.312°C; the melting temperature of the PCR product with G at 97th position is 66.551°C. The blue curve represents negative control (water).

As shown in Figure 6.74, the fluorescence intensity of the PCR product decreases sharply at the melting temperature. According to the data analysis based on the Figure 6.75, The melting temperature of the PCR product with A at 97th position is located at 66.312°C, while the melting temperature of the PCR product with G at 97th position is located at 66.551°C. The difference of melting temperature between them is 0.239°C. It is only 9.08% offset to the theoretical value (0.217) obtained from the formula (6.1). Thus above real 189 base-pair PCR products with only one base pair (A/G) difference can be successfully detected using chip system.

Based on a number of DNA melting experiments, the melting temperature scan and detection resolutions can be controlled by various temperature scan rate, as shown in Table 6.5. Thus precise DNA melting curve analysis is achieved on the chip system successfully.

Table 6.5: Specifications of DNA melting analysis experiments

Temperature scan resolution	Temperature scan rate	Total time (from 60°C to 95°C)
0.01°C	0.02°C/s	1750 second
0.1°C	0.2°C/s	175 second
0.5°C	1°C/s	35 second

6.7 Summary

Based on above experiments, the PCR results on chip system was compared among air flow PCR machine, TEC PCR machine and commercial machine (Rotor gene PCR machine). The thermal control experiments are verified similar with the simulation results, and the biological experiments are proved to be successful in trial. Both of the

thermal-experiment and the bio-experiment are better than other systems. Rapid (8 second) and multiple (as a new function) PCR is realized on chip PCR system.

The superior experiment results of DNA melting curve analysis can also be achieved on chip system. High precision and uniformity are guaranteed by conducting the thermal experiments. In biological experiment, only one base pair difference in DNA sequence would be detected clearly, which is impossible by other systems.

Chapter 7 Improvements and future work

7.1 Improvements

7.1.1 Loading and sealing

In the chip system, the PCR sample is loaded and sealed manually, which would cause inconvenience and contamination during experiments. For high density chamber array and high throughput, manual operation is incompatible. To improve the sealing method, some investigation is carried out in this section.

A micro-fluidic sealing method is on trial developed by the research group of BioMEMS lab in NTU (shown as Figure 7.1). Surface tension effects are dominant at small length scales prevalent in micro-fluidic devices. Hydrophobic surfaces can be manipulated to have a passive valve (because of an absence of moving parts) and are being increasingly used for flow control in the micro-bio-systems. [100]

Figure 7.1: Photomask and the definition of the design.

EGC-1700 Hydrophobic Coating Liquid was applied onto outlet channel of the micro-fluid chip, thus the outlet channel became hydrophobic. The micro-fluidic flow pattern of sample loading into reaction micro-well is shown as following Figures.

Figure 7.2: Photos of sample loading. (a) The sample starts flowing into the 1st Micro-well from Inlet Channel through the Inlet Bridge Channel. (b) The sample starts to fill up the 1st Micro-well while it is also travels along the Inlet Channel towards the 2nd Micro-well. (c) The sample fully filled up the 1st Micro-well without overflowing. (d) The sample starts flowing into the 2nd Micro-well while it is traveling towards the 3rd Well through the Inlet Channel. (e) The sample fully filled up the 2nd Micro-well and starts to flow into the 3rd Micro-well. (f) The sample is filling up the 3rd Micro-well. The meniscus shows the velocity profile when the sample flows into the Micro-well. The maximum flow velocity occurs at the center. (g) The 3rd Micro-well is half filled now. The meniscus becomes more flatten (horizontal). This implies that the velocity profile is quite uniform now. The flow velocity

is about the same at the center and the side. (h) The sample fully filled up all the three Micro-wells. EGC-1700 at the Outlet Channel is able to successfully prevent overflow of sample.

After loading sample into the PCR reaction well,

Figure 7.3: Photos of sample sealing. (a) The sealing oil (transparent) pushes the sample (red colour), which is in the Inlet Channel, towards the waste without flowing into the Micro-wells. (b) The sealing oil has fully sealed up the Inlet Channel. (c) After a while, the sealing oil slightly went into the Micro-well. (d) The sealing oil (transparent) is slowly sealing up the Outlet Channel. (e) The sealing oil has fully sealed up the Outlet Channel.

Shown as Figure 7.2 and 7.3, the sample loading and sealing is successful. More investigations and testing are carried out till now. If this micro fluidic design is combined with previous chip design, the operation of the microchip system would be simple and convenient definitely.

7.1.2 Groove design

A groove structure in the PCR improves the performance of DNA amplification. The effects of groove geometry, including width, depth and position, on the thermal characteristics of the PCR chip have been investigated by numerical analysis and experimental measurement [101].

Figure 7.4: Cross-sectional view of the PCR chip with two grooves [101]

The PCR heating rate is increased rapidly with the increase of the groove depth, shown as Figure.

Figure 7.5: Variations of the chamber temperature during the heating stage depending on the groove depth [101]

Figure shows the temperature variations measured depending on the groove depth. The deeper the grooves, the higher the heating rates. This conclusion is conducted in the experiments, shown as Figure 7.6.

Figure 7.6: Heating profiles of the chip with different groove depths [101]

But the above investigation is based on a chip with 3.6 μ l PCR chamber and 17 $\times$ 40mm² Si-based micromachined structure, which is much larger than the microchip described in previous chapters. It must also be considered that the yield strength, fabricate operation and life duration would be worse with the increase of groove depth and width. Although it is believed that the groove in the chip will improve the PCR performance definitely, the effect of this design should be re-investigated and re-evaluated.

7.2 Summary and conclusions

7.2.1 Summary

In the present research work, a PCR chamber array on a single microchip was designed and investigated based on the thermal processes required by the PCR

reaction and the DNA melting analysis. With PCR samples much smaller (at least 100 times or more) than the volume required in conventional PCR devices and thin film micro heaters and RTD sensors, the time for completing a PCR and the costs for expensive PCR reagents can be greatly reduced using the proposed microchip. Since the minimized size and perfect temperature control of the PCR chip, every chamber in this chip can be controlled independently without thermal cross-talk. During the DNA melting curve analysis process, this chip design realized precise temperature control (0.01°C resolution) and good temperature uniformity (less than 0.001°C difference).

Both PCR process and DNA melting curve analysis process in the expected chip have been verified and supported by 2-D and 3-D modelings, followed by FEM and CFD simulations. After microfabricated by MEMS technologies, the chip was tested with the actual thermal conditioning, which is similar with the simulation results. More experiments are carried out to investigate the performance of different chip with different chamber characteristics. As the final step, bio-experiments are conducted in this chip system. Rapid PCR (comparing with Rotor-gene), multiple PCR (each chamber can be controlled independently), and precise DNA melting curve analysis are successfully achieved on this chip.

Thus “a lab in a chip” is realized. The integration of PCR and DNA melting analysis into one chip as described in this work opened a new field of research and application in biology.

7.2.2 Conclusions

Based on the work done, the following conclusions can be drawn:

- The analytical models developed in present work can be applied to study PCR thermal cycling characteristics of the chips from conduction and convection heat transfer. The 2-D and 3-D FEM simulations using ANSYS (Version 6.1) were established to supplement the analytical models and provide chip design principles.

- Based on both analytical solutions and FEM simulation results, the proposed design was capable of attaining rapid thermal cycling and uniform temperature in the PCR process and precise temperature scan control in the DNA melting analysis.
- It was found that the nano-litre reaction chamber in the proposed chip resulted in a fast thermal cycling and precise temperature scan control. Furthermore, a high throughput PCR capability could be achieved through the microfabricated PCR chamber array.
- Based on the current MEMS technology, the microfabrication processes for fabricating the proposed chip were established. Prototypes of PCR chip are fabricated.
- Experiments on a microchip PCR system and the reference systems were setup and tested for a rapid thermal cycling successfully. The thermal properties of the chip PCR system are investigated and perfectly accord with the simulation results. The two reference systems, the air PCR system and the TEC PCR system, are able to generate expected thermal cycling performances.
- Experiments on the chip system for DNA melting curve analysis were setup and tested successfully. The thermal properties of the chip PCR system are investigated and perfectly accord with the simulation results.
- Successful PCR products detected. PCR mixture of the same batch is used to run in other commercial PCR machines and the chip PCR system. Their results are compared, and the cycle of amplification coincides. Hence, the PCR chip has performed PCR successfully.
- Real-time PCRs with reversed thermal cycling profiles are performed successfully on the chip. The PCR product of each PCR element is detected. Multiple PCR reactions in the PCR chamber array are achieved independently and simultaneously.
- Precise temperature control for DNA melting curve analysis was obtained successfully. DNA templates with one base pair difference were detected on the chip.

7.3 Future work

7.3.1 Fine-tuning of the PCR system to achieve greater stability and reliability

Till now, though it has higher detection resolution, the chip system for DNA melting curve analysis is not as stable and reliable as a commercial machine. The main cause for this condition is that this system is too sensitive to detect any slight change of its surroundings; even a breeze might import temperature information unnecessary. Current resolution is using a good isolation material (such as plastic) to fabricate a isolated chip container. However, a better method is to control the air temperature inside the container simultaneously. Hence, a small oven is fabricated to protect the micro heater and micro sensor from variable surrounding more efficiently.

7.3.2 Design of high density channel array

Since one chip containing four PCR chamber is tested successfully, PCR chip with high density channel array is required in the future. Less volume, less size but high resolution detection system allows this idea to come into true. With the improved sealing method, all the PCR chambers can be designed as PCR channels. If these channels are 1mm(length) $\times$ 0.2mm(width), about 1500 samples can be performed DNA amplification and detection simultaneously in a 26mm $\times$ 72mm glass slide. Thus, high quantity clinical experiments would be realized in the following years.

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Appendix A Calculation for convection heat transfer coefficient

Assumptions

- Material has constant properties
- Negligible buoyancy effects
- Negligible surface radiation effects
- Uniform surface temperature, T_s
- Uniform ambient temperature, T_∞

Known information

Density of glass, $\rho = 2250 \text{ kg} / \text{m}^3$

Specific heat capacity of glass, $c = 700 \text{ J} / \text{kg} \cdot \text{K}$

Steady state:

Calculations for convection coefficients during denaturation stage 2 (95 °C)

Since ambient air heats up at a slower rate as compared to the chip element by conduction, therefore it is assumed the ambient air temperature, T_∞ , as the room temperature (25°C) and the surface temperatures of the plate T_s is 95°C during denaturation process.

But the reference air temperature, $T_f = \frac{1}{2}(368+298) = 333 \text{ K}$ (60 °C)

Obtaining the following data from Table 1 in Appendix B

From properties of air at $T_f = 333 \text{ K}$ from Appendix B,

Kinematic viscosity, $\nu = 19.21 \times 10^{-6} \text{ m}^2/\text{s}$

Prandtl number, $\text{Pr} = 0.702$

Expansion coefficient, $\beta = 1/333 = 3.003 \times 10^{-3} \text{ K}^{-1}$

Thermal diffusivity, $\alpha = 27.4 \times 10^{-6} \text{ m}^2 / \text{s}$

Thermal conductivity, $k = 28.7 \times 10^{-3} \text{ W/mK}$

The following empirical equations [95] are employed in the present calculation

Appendix A Calculation for convection heat transfer coefficient

Reynolds number,
$$Re_L = \frac{VL_{chip}}{\nu}$$

Where V is the air flow velocity and L_{chip} is the characteristic length of the PCR Microchip.

For the top and bottom plates, because the air channel is not at the margin of the chip, so the average length $L_{chip}=4.5+3.2=7.7\text{mm}$

For the inside surface, the average length $L_{chip}=3.2+3.2=6.4\text{mm}$

Convection coefficient for top and bottom horizontal surface during Process 2

$$Re_L = \frac{VL_{chip}}{\nu} = \frac{V(7.7 \times 10^{-3})}{19.21 \times 10^{-6}} = 400.833V \quad (\text{Since } < 5 \times 10^5, \text{ laminar flow})$$

$$\text{Nusselt number, } \overline{Nu}_L = 0.664 Re_L^{1/2} Pr^{1/3} = 11.815V^{1/2}$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L} = 44.04V^{1/2} \left(\frac{W}{m^2 \cdot K} \right)$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 88.08 \left(\frac{W}{m^2 \cdot K} \right)$$

Convection coefficient for inside surface during Process 2

$$Re_L = \frac{VL_{chip}}{\nu} = \frac{V(6.4 \times 10^{-3})}{19.21 \times 10^{-6}} = 333.160V \quad (\text{Since } < 5 \times 10^5, \text{ laminar flow})$$

$$\text{Nusselt number, } \overline{Nu}_L = 0.664 Re_L^{1/2} Pr^{1/3} = 10.771V^{1/2}$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L} = 40.15V^{1/2} \left(\frac{W}{m^2 \cdot K} \right)$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 80.30 \left(\frac{W}{m^2 \cdot K} \right)$$

Calculations for convection coefficients during annealing stage 4 (55 °C)

Since ambient air heats up at a slower rate as compared to the chip element by conduction, therefore it is assumed the ambient air temperature, T_∞ , as the average of the room temperature (25 °C) and the surface temperatures of the plate is 55 °C during denaturation process.

The reference air temperature, $T_f = \frac{1}{2}(328+298) = 313 \text{ K (40 °C)}$

Obtaining the following data from Table 1 in Appendix B

From properties of air at $T_f = 313\text{K}$ from Appendix B,

Appendix A Calculation for convection heat transfer coefficient

Kinematic viscosity, $\nu = 17.20 \times 10^{-6} \text{ m}^2/\text{s}$

Prandtl number, $\text{Pr} = 0.705$

Expansion coefficient, $\beta = 1/313 = 3.195 \times 10^{-3} \text{ K}^{-1}$

Thermal diffusivity, $\alpha = 24.4 \times 10^{-6} \text{ m}^2/\text{s}$

Thermal conductivity, $k = 29.0 \times 10^{-3} \text{ W/m}\cdot\text{K}$

Convection coefficient for top and bottom horizontal surface during Process 4

$$\text{Re}_L = \frac{VL_{chip}}{\nu} = \frac{V(7.7 \times 10^{-3})}{17.20 \times 10^{-6}} = 447.674V \quad (\text{Since } < 5 \times 10^5, \text{ laminar flow})$$

$$\text{Nusselt number, } \overline{Nu}_L = 0.664 \text{Re}_L^{1/2} \text{Pr}^{1/3} = 12.504V^{1/2}$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L} = 47.09V^{1/2} \left(\frac{\text{W}}{\text{m}^2 \cdot \text{K}} \right)$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 94.18 \left(\frac{\text{W}}{\text{m}^2 \cdot \text{K}} \right)$$

Convection coefficient for inside surface during Process 4

$$\text{Re}_L = \frac{VL_{chip}}{\nu} = \frac{V(6.4 \times 10^{-3})}{17.20 \times 10^{-6}} = 372.093V \quad (\text{Since } < 5 \times 10^5, \text{ laminar flow})$$

$$\text{Nusselt number, } \overline{Nu}_L = 0.664 \text{Re}_L^{1/2} \text{Pr}^{1/3} = 11.400V^{1/2}$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L} = 51.65V^{1/2} \left(\frac{\text{W}}{\text{m}^2 \cdot \text{K}} \right)$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 103.30 \left(\frac{\text{W}}{\text{m}^2 \cdot \text{K}} \right)$$

Calculations for convection coefficients during extension stage 6 (70 °C)

Since ambient air heats up at a slower rate as compared to the chip element by conduction, therefore it is assumed the ambient air temperature, T_∞ , as the average of the room temperature (25 °C) and the surface temperatures of the plate is 70 °C during denaturation process.

The reference air temperature, $T_f = \frac{1}{2}(343+298) = 320.5 \text{ K}$ (47.5 °C)

Obtaining the following data from Table 1 in Appendix B

From properties of air at $T_f = 320.5\text{K}$ from Appendix B,

Kinematic viscosity, $\nu = 17.95 \times 10^{-6} \text{ m}^2/\text{s}$

Prandtl number, $\text{Pr} = 0.704$

Appendix A Calculation for convection heat transfer coefficient

Expansion coefficient, $\beta = 1/320.5 = 3.120 \times 10^{-3} \text{K}^{-1}$

Thermal diffusivity, $\alpha = 25.5 \times 10^{-6} \text{m}^2/\text{s}$

Thermal conductivity, $k = 27.8 \times 10^{-3} \text{W/m}\cdot\text{K}$

Convection coefficient for top and bottom horizontal surface during Process 4

$$\text{Re}_L = \frac{VL_{chip}}{\nu} = \frac{V(7.7 \times 10^{-3})}{17.95 \times 10^{-6}} = 428.969V \quad (\text{Since } < 5 \times 10^5, \text{ laminar flow})$$

$$\text{Nusselt number, } \overline{Nu}_L = 0.664 \text{Re}_L^{1/2} \text{Pr}^{1/3} = 12.240V^{1/2}$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L} = 44.19V^{1/2} \left(\text{W}/\text{m}^2 \cdot \text{K} \right)$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 88.38 \left(\text{W}/\text{m}^2 \cdot \text{K} \right)$$

Convection coefficient for inside surface during Process 4

$$\text{Re}_L = \frac{VL_{chip}}{\nu} = \frac{V(6.4 \times 10^{-3})}{17.95 \times 10^{-6}} = 356.546V \quad (\text{Since } < 5 \times 10^5, \text{ laminar flow})$$

$$\text{Nusselt number, } \overline{Nu}_L = 0.664 \text{Re}_L^{1/2} \text{Pr}^{1/3} = 11.159V^{1/2}$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L} = 48.47V^{1/2} \left(\text{W}/\text{m}^2 \cdot \text{K} \right)$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 96.94 \left(\text{W}/\text{m}^2 \cdot \text{K} \right)$$

Unsteady state:

Calculations for convection coefficients during temperature transition stage 1

Since ambient air heats up at a slower rate as compared to the chip element by radiation, therefore we assume the ambient air temperature, T_∞ , as the room temperature, 25°C , 298K . Assuming the surface temperature, T_s , as the average value of the initial (70°C) and final temperature (95°C) during process 1.

$$\text{i.e. surface temperature, } T_s = \frac{1}{2}(343 + 368) = 355.5 \text{ K}$$

Obtaining the following data from Table 1 in Appendix B

All properties are evaluated at film temperature, i.e. $T_f = \frac{1}{2}(298 + 355.5) = 326.75 \text{ K}$

From properties of air at $T_f = 326.75\text{K}$ from Appendix B,

Appendix A Calculation for convection heat transfer coefficient

Kinematic viscosity, $\nu = 18.58 \times 10^{-6} \text{ m}^2/\text{s}$

Prandtl number, $\text{Pr} = 0.703$

Expansion coefficient, $\beta = 1/323 = 3.060 \times 10^{-3} \text{ K}^{-1}$

Thermal diffusivity, $\alpha = 26.459 \times 10^{-6} \text{ m}^2/\text{s}$

Thermal conductivity, $k = 28.3 \times 10^{-3} \text{ W/m}\cdot\text{K}$

The following empirical equations are employed in the present calculation

$$\text{Rayleigh number, } \text{Ra} = \frac{g\beta(T_s - T_\infty)L_c^3}{\alpha\nu} = 3.507 \times 10^9 \quad L_c^3 \quad (10^7 \leq \text{Ra}_L \leq 10^{11})$$

Where L_c is the characteristic length of the PCR microchip.

$$\text{Nusselt number, } \overline{\text{Nu}}_L = 0.15 \text{Ra}_L^{1/3} = 227.906 \quad L_c$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{\text{Nu}}_L \cdot k}{L_c} = 6.450 \left(\frac{\text{W}}{\text{m}^2 \cdot \text{K}} \right)$$

Calculations for convection coefficients during temperature transition stage 3

It is assumed the ambient air temperature, T_∞ , as the room temperature, 25°C , 298K .

Assuming the surface temperature, T_s , as the average value of the initial (95°C) and final temperature (55°C) during process 1.

$$\text{i.e. surface temperature, } T_s = \frac{1}{2}(328 + 368) = 348 \text{ K}$$

Obtaining the following data from Table 1 in Appendix B

All properties are evaluated at film temperature, $T_f = \frac{1}{2}(348 + 298) = 323 \text{ K}$

From properties of air at $T_f = 323 \text{ K}$ from Appendix B,

Kinematic viscosity, $\nu = 18.2038 \times 10^{-6} \text{ m}^2/\text{s}$

Prandtl number, $\text{Pr} = 0.7038$

Expansion coefficient, $\beta = 1/323 = 3.0960 \times 10^{-3} \text{ K}^{-1}$

Thermal diffusivity, $\alpha = 25.9040 \times 10^{-6} \text{ m}^2/\text{s}$

Thermal conductivity, $k = 28.0020 \times 10^{-3} \text{ W/m}\cdot\text{K}$

The following empirical equations are employed in the present calculations [49].

$$\text{Reynolds number, } \text{Re}_L = \frac{VL_{chip}}{\nu}$$

Where V is the air flow velocity and L_{chip} is the length of the PCR microchip.

Appendix A Calculation for convection heat transfer coefficient

Convection coefficient for top and bottom horizontal surface at Process 3

$$\text{Re}_L = \frac{VL_{chip}}{\nu} = \frac{V(7.7 \times 10^{-3})}{18.2038 \times 10^{-6}} = 422.989V \quad (\text{Since } < 5 \times 10^5, \text{ laminar flow})$$

$$\text{Nusselt number, } \overline{Nu}_L = 0.664 \text{Re}_L^{1/2} \text{Pr}^{1/3} = 12.147V^{1/2}$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L} = 44.175V^{1/2}$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 88.35 \left(\frac{\text{W}}{\text{m}^2 \cdot \text{K}} \right)$$

Convection coefficient for inside surface at Process 3

$$\text{Re}_L = \frac{VL_{chip}}{\nu} = \frac{V(6.4 \times 10^{-3})}{18.2038 \times 10^{-6}} = 351.575V \quad (\text{Since } < 5 \times 10^5, \text{ laminar flow})$$

$$\text{Nusselt number, } \overline{Nu}_L = 0.664 \text{Re}_L^{1/2} \text{Pr}^{1/3} = 11.074V^{1/2}$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L} = 48.45V^{1/2}$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 96.90 \left(\frac{\text{W}}{\text{m}^2 \cdot \text{K}} \right)$$

Calculation for convection coefficient during temperature transition stage 5

It is assumed the ambient air temperature, T_∞ , as the room temperature, 25 °C, 298K.

Assuming the surface temperature, T_s , as the average value of the initial (55 °C) and final temperature (70 °C) during process 5.

$$\text{i.e. surface temperature, } T_s = \frac{1}{2}(343 + 328) = 335.5 \text{ K}$$

Obtaining the following data from Table 1 in Appendix B

All properties are evaluated at film temperature, i.e. $T_f = \frac{1}{2}(298 + 335.5) = 316.75 \text{ K}$

From properties of air at $T_f = 316.75 \text{ K}$ from Appendix B,

Kinematic viscosity, $\nu = 17.5751 \times 10^{-6} \text{ m}^2/\text{s}$

Prandtl number, $\text{Pr} = 0.7047$

Expansion coefficient, $\beta = 1/316.75 = 3.1571 \times 10^{-3} \text{ K}^{-1}$

Thermal diffusivity, $\alpha = 24.9790 \times 10^{-6} \text{ m}^2/\text{s}$

Thermal conductivity, $k = 27.5395 \times 10^{-3} \text{ W/m}\cdot\text{K}$

Appendix A Calculation for convection heat transfer coefficient

The following empirical equations are employed in the present calculation

$$\text{Rayleigh number, } Ra = \frac{g\beta(T_s - T_\infty)L_c^3}{\alpha\nu} = 2.643 \times 10^9 L_c^3 \quad (10^7 \leq Ra_L \leq 10^{11})$$

Where L_c is the characteristic length of the PCR microchip.

$$\text{Nusselt number, } \overline{Nu}_L = 0.15 Ra_L^{1/3} = 207.387 L_c$$

$$\text{Average convection coefficient, } \bar{h} = \frac{\overline{Nu}_L \cdot k}{L_c} = 5.711 \left(\frac{W}{m^2 \cdot K} \right)$$

Appendix B Air Thermophysical property table

Appendix B Air Thermophysical property table

Thermophysical Properties of Gases at Atmospheric Pressure^a

T (K)	ρ (kg/m ³)	c_p (kJ/kg · K)	$\mu \cdot 10^7$ (N · s/m ²)	$\nu \cdot 10^6$ (m ² /s)	$k \cdot 10^3$ (W/m · K)	$\alpha \cdot 10^6$ (m ² /s)	Pr
Air							
100	3.5562	1.032	71.1	2.00	9.34	2.54	0.786
150	2.3364	1.012	103.4	4.426	13.8	5.84	0.758
200	1.7458	1.007	132.5	7.590	18.1	10.3	0.737
250	1.3947	1.006	159.6	11.44	22.3	15.9	0.720
300	1.1614	1.007	184.6	15.89	26.3	22.5	0.707
350	0.9950	1.009	208.2	20.92	30.0	29.9	0.700
400	0.8711	1.014	230.1	26.41	33.8	38.3	0.690
450	0.7740	1.021	250.7	32.39	37.3	47.2	0.686
500	0.6964	1.030	270.1	38.79	40.7	56.7	0.684
550	0.6329	1.040	288.4	45.57	43.9	66.7	0.683
600	0.5804	1.051	305.8	52.69	46.9	76.9	0.685
650	0.5356	1.063	322.5	60.21	49.7	87.3	0.690
700	0.4975	1.075	338.8	68.10	52.4	98.0	0.695
750	0.4643	1.087	354.6	76.37	54.9	109	0.702
800	0.4354	1.099	369.8	84.93	57.3	120	0.709
850	0.4097	1.110	384.3	93.80	59.6	131	0.716
900	0.3868	1.121	398.1	102.9	62.0	143	0.720
950	0.3666	1.131	411.3	112.2	64.3	155	0.723
1000	0.3482	1.141	424.4	121.9	66.7	168	0.726
1100	0.3166	1.159	449.0	141.8	71.5	195	0.728
1200	0.2902	1.175	473.0	162.9	76.3	224	0.728
1300	0.2679	1.189	496.0	185.1	82	238	0.719
1400	0.2488	1.207	530	213	91	303	0.703
1500	0.2322	1.230	557	240	100	350	0.685
1600	0.2177	1.248	584	268	106	390	0.688
1700	0.2049	1.267	611	298	113	435	0.685
1800	0.1935	1.286	637	329	120	482	0.683
1900	0.1833	1.307	663	362	128	534	0.677
2000	0.1741	1.337	689	396	137	589	0.672
2100	0.1658	1.372	715	431	147	646	0.667
2200	0.1582	1.417	740	468	160	714	0.655
2300	0.1513	1.478	766	506	175	783	0.647
2400	0.1448	1.558	792	547	196	869	0.630
2500	0.1389	1.665	818	589	222	960	0.613
3000	0.1135	2.726	955	841	486	1570	0.536

Table 1: Thermophysical property of air at atmospheric pressure [86]

Appendix C Summary of heat transfer methods

Discipline of heat transfer is concerned with only two things: temperature and flow of heat. Temperature represents the amount of thermal energy available, while heat flow represents the movement of thermal energy from place to place. On a microscopic scale, thermal energy is related to the kinetic energy of molecules. It is natural for regions containing greater molecular kinetic energy to pass this energy to regions with less kinetic energy. Several material properties serve to modulate the heat transferred between two regions at different temperature. Examples include thermal conductivities, specific heats, material densities, fluid velocities, fluid viscosities, surface emissivities, and so on. There are three broad categories of heat transfer mechanisms, namely conduction, convection and radiation.

1 Conduction heat transfer in PCR microchips

When there is a temperature gradient in a body, heat energy will flow from the region of high temperature to the region of low temperature. This phenomenon is known as conduction heat transfer, and is described by Fourier's Law, $q = -k\nabla T$ (1)

This equation determines heat flux vector q for a given temperature profile T and thermal conductivity k . The minus sign ensures that heat flows down the temperature gradient. Heat conduction is the simplest and most directly method to transfer heat to PCR sample, but it is also the most efficient one.

Thermal mass between sample and heat source is main restricting factor to achieve rapid ramping rate and precise temperature control. Thus, in most commercially micro PCR instrumentations, heating/cooling block is usually in intimate contact with PCR reaction chamber to reduce thermal mass between them. Now with development of MEMS technique, it can be achieved easily.

2 Convection heat transfer

Heat energy transfers between a solid and a fluid when there is a temperature difference between the fluid and the solid. This is known as "convection heat transfer". The essential ingredients of forced convection heat transfer analysis are

given by Newton's Law of Cooling; $\dot{Q} = hA(T_w - T_\infty) = hA \cdot \Delta T$ (2)

The rate of heat $\dot{Q}$ transferred to the surrounding fluid is proportional to object's exposed area A , and difference between object temperature T_w and fluid free-stream temperature T_∞ . The constant of proportionality h is termed as convection heat-transfer coefficient.

In convection model of flow over flat plate, viscosity of the fluid requires that fluid have zero velocity at plate's surface, thus a boundary layer exists where the fluid velocity changes from u_∞ in the free stream (far from the plate) to zero at the plate. Within this boundary layer, the flow is initially laminar but can proceed to turbulence once the Reynolds Number Re of the flow is sufficiently high.

The transition from laminar to turbulent for flow over a flat plate occurs in the range,

$$3 \times 10^5 < Re_x < 3 \times 10^6, \quad Re_x = \frac{\rho u_\infty x}{\mu} \quad (3)$$

Thus, laminar flow will have $Re_x < 3 \times 10^5$, while turbulent flow occurs when $Re_x > 3 \times 10^6$. The following correlations employ a number of dimensionless parameters to describe convection heat transfer.

General Convection (Forced and Free)

Table 1: Dimensionless parameters for both forced and free convections

Parameter	Formula	Interpretation
Prandtl Number:	$Pr = \frac{\nu}{\alpha} = \frac{c_p \mu}{k}$	Ratio of fluid velocity boundary layer thickness to the fluid temperature boundary layer thickness.

Nusselt Number:	$Nu = \frac{hL}{k}$	Ratio of heat transferred from surface to heat conducted away by fluid.
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Forced Convection Only

Table 2: Dimensionless parameters for forced convection

Parameter	Formula	Interpretation
Reynolds Number:	$Re_L = \frac{u_\infty L}{\nu} = \frac{\rho u_\infty L}{\mu}$	Ratio of fluid inertia stress to viscous stress (for flow over flat plates).
Reynolds Number:	$Re_D = \frac{u_\infty D}{\nu} = \frac{\rho u_\infty D}{\mu}$	(Reynolds Number for pipe flow).

Free Convection Only

Table 3: Dimensionless parameters for free convection

Parameter	Formula	Interpretation
Grashof Number:	$Gr = \frac{g\beta \Delta T L^3}{\nu^2}$	Ratio of fluid buoyancy stress to viscous stress.
Rayleigh Number:	$Ra_L = Gr \cdot Pr = \frac{g\beta(T_s - T_\infty)L^3}{\nu\alpha}$	

Where L is characteristic length of geometry, α is thermal diffusivity, $\beta = 1/T_f$, g is gravitational acceleration and ν is viscosity. Typically, $n = 1/4$ for laminar flow. The average of wall and free-stream temperatures, defined as film temperature T_f .

Correlations for Nusselt number and Rayleigh number is as following equations.

$$\overline{Nu}_L = 0.54 Ra_L^{1/4}, \quad 10^4 \leq Ra_L \leq 10^7 \quad (4)$$

$$\overline{Nu}_L = 0.15 Ra_L^{1/3}, \quad 10^7 \leq Ra_L \leq 10^{11} \quad (5)$$

PCR Thermal cycling by convection is usually achieved by heating/cooling a circulating medium such as air to affect the temperature of sample. Convection coefficient of forced air is much higher than free air. Forced air at room temperature

flowing through the reaction chamber is an efficient method for cooling. But the ambient temperature restricts the maximum or minimum temperature at which the device can operate. Control of ambient temperature would reduce this restriction.

3 Radiation heat transfer

The radiation heat transfer is described by the Stefan-Boltzmann Law

$$Q = \varepsilon A \sigma (T^4 - T_e^4) \quad (6)$$

where Q is the heat transfer, ε is the surface emissivity, A is the total radiating area, σ is the Stefan-Boltzmann constant, T is the temperature of body, T_e is the temperature of surroundings. Using radiation heat transfer in PCR microchip applications could be manipulated easily. Heating by radiation can mainly focus on affecting the sample itself, rather than the reaction vessel and its environment. But the heating effect is deeply influenced by the absorptivity of PCR sample. In this work, the total radiation heat transfer from PCR microchip to surroundings is in the order of 10^{-5} , which is extremely small compared with the other two heat transfer methods. Thus the radiation heat transfer is neglected in the PCR microchip.

Appendix D Analytical solution of thermal diffusion problem in composite layers

The model for transient temperature distribution of one layer [69, 95] is illustrated in Figure 1.

Figure 1: Thermal diffusion model in one plate. Where L is the thickness of the layer, the top boundary is assumed as a thermal insulation boundary and the temperature of bottom boundary switches from T_0 to T_1 at the initial time.

$$\frac{\partial T}{\partial t} = a^2 \frac{\partial^2 T}{\partial x^2} \quad (0 < x < L, t > 0) \quad (1)$$

$$T(x, 0) = T_0 \quad (0 \leq x \leq L) \quad (2)$$

$$\left. \frac{\partial T(x, t)}{\partial x} \right|_{x=L} = 0 \quad (3)$$

$$T(0, t) = T_1 \quad (0 < t) \quad (4)$$

where $a^2 = \frac{K}{\sigma \rho}$, a^2 is the thermal diffusivity, K the thermal conductivity, σ the specific heat, and ρ the density of the material of the layer.

For (1), set $T(x, t) = \mu(x, t) + \psi(x)$

$$\Rightarrow \frac{\partial \mu}{\partial t} = a^2 \frac{\partial^2 \mu}{\partial x^2} + a^2 \psi''(x)$$

For (4), $T(0, t) = \mu(0, t) + \psi(0) = T_1$

set $\psi''(x) = 0; \psi(0) = T_1$

$$\psi(x) = cx + d$$

$$\psi(0) = d = T_1$$

$$\text{For (3), } \frac{\partial T(L, t)}{\partial x} = \frac{\partial \mu(L, t)}{\partial x} + \psi'(L) = 0$$

$$\psi'(0) = 0; \Rightarrow c = 0; \psi(x) = T_1$$

$$\text{So, } \frac{\partial \mu}{\partial t} = a^2 \frac{\partial^2 \mu}{\partial x^2} \quad (5)$$

$$\mu(0, t) = 0 \quad (6)$$

$$\frac{\partial \mu(L, t)}{\partial t} = 0 \quad (7)$$

$$\mu(x, 0) + T_1 = T_0 \quad (8)$$

$$\text{set } \mu(x, t) = X(x)U(t)$$

$$U'X = a^2 X''U$$

$$\frac{X''}{X} = \frac{1}{a^2} \frac{U'}{U} = \lambda$$

$$\text{Set } \lambda < 0; (\lambda = -p^2)$$

$$\text{then, } X'' + p^2 X = 0; m^2 + p^2 = 0; m = \pm ip$$

$$X(x) = A \cos(px) + B \sin(px)$$

$$\text{For (6), } X(0) = A = 0$$

$$\mu(x, t) = B \sin(px)U(t)$$

$$\text{For (7), } \frac{\partial \mu(L, t)}{\partial t} = pB \cos(pL)U(t) = 0$$

$$\cos(pL) = 0$$

$$pL = \frac{n\pi}{2}, n = (2k-1), k = 1, 2, 3, \dots$$

$$p = \frac{n\pi}{2L}$$

$$\text{so, } X_n(x) = B_n \sin\left(\frac{n\pi}{2L}x\right)$$

$$\text{Cause, } U' + (pa)^2 U = 0$$

$$\ln U = -(pa)^2 t + d$$

$$U_n(t) = C_n e^{-\left(\frac{n\pi a}{2L}\right)^2 t}$$

$$\text{so, } \mu_n(x, t) = X_n(x)U_n(t) = D_n e^{-\left(\frac{n\pi a}{2L}\right)^2 t} \sin\left(\frac{n\pi}{2L} x\right)$$

$$\text{where, } D_n = C_n B_n$$

$$\mu(x, t) = \sum_{n=1}^{\infty} \mu_n(x, t)$$

$$\begin{aligned} \text{For (8), } \sum_{n=1}^{\infty} D_n \sin\left(\frac{n\pi}{2L} x\right) &= T_0 - T_1 \\ D_n &= \frac{2}{L} \int_0^L (T_0 - T_1) \sin\left(\frac{n\pi x}{2L}\right) dx = \frac{4(T_1 - T_0)}{n\pi} \left[\cos\left(\frac{n\pi}{2}\right) - 1\right] \end{aligned}$$

$$\mu(x, t) = \sum_{n=1}^{\infty} \frac{4(T_1 - T_0)}{n\pi} \left[\cos\left(\frac{n\pi}{2}\right) - 1\right] e^{-\left(\frac{n\pi a}{2L}\right)^2 t} \sin\left(\frac{n\pi}{2L} x\right)$$

$$T(x, t) = \mu(x, t) + \psi(x) = \mu(x, t) + T_1$$

$$\text{so, } = \sum_{n=1}^{\infty} \frac{4(T_1 - T_0)}{n\pi} \left[\cos\left(\frac{n\pi}{2}\right) - 1\right] e^{-\left(\frac{n\pi a}{2L}\right)^2 t} \sin\left(\frac{n\pi}{2L} x\right) + T_1$$

where a^2 is the thermal diffusivity, T_0 is the initial temperature of the heater, T_1 is the final temperature of the heater, L is the thickness of the layer, t is the time.

The actual condition in PCR microchip can be simplified as a two composite walls problem illustrated as Figure 2.

Figure 3.6: Thermal diffusion model of two composite walls, where L_1 is the thickness of sample layer, L_2 is the thickness of glass layer, a^2 is the thermal diffusivity of sample layer, b^2 is the thermal diffusivity of glass layer, and arrows are the direction of heat transfer. SU-8 cover is assumed as a thermal insulation boundary. At the initial time, the temperature of heater increases from T_0 to T_1 .

Similar with the results of the one layer model [70, 71], the temperature of the top surface becomes

$$T_{top} = \sum_{n=1}^{\infty} \frac{4(T_1 - T_0)}{n\pi} [\cos(\frac{n\pi}{2}) - 1] e^{-\left(\frac{n\pi a}{2(L_1 + a\frac{L_2}{b})}\right)^2 t} \sin(\frac{n\pi}{2}) + T_1$$

And the temperature of the bottom surface becomes

$$T_{bottom} = \sum_{n=1}^{\infty} \frac{4(T_1 - T_0)}{n\pi} [\cos(\frac{n\pi}{2}) - 1] e^{-\left(\frac{n\pi a}{2L_2\frac{a}{b}}\right)^2 t} \sin(\frac{n\pi}{2}) + T_1$$

$$\text{set } \theta = \frac{4(T_1 - T_0)}{n\pi} [\cos(\frac{n\pi}{2}) - 1] e^{-\lambda t} \sin(\frac{n\pi}{2}),$$

where λ is a constant.

If $n = 1$,

$$\theta_1 = -\frac{4(T_1 - T_0)}{\pi} e^{-\lambda t}$$

If $n > 1$,

$$\theta_n = \frac{4(T_1 - T_0)}{n\pi} [\cos(\frac{n\pi}{2}) - 1] e^{-\lambda t} \sin(\frac{n\pi}{2})$$

which is much less than θ_1 ;

So an approximation can be made:

$$\sum_{n=1}^{\infty} \frac{4(T_1 - T_0)}{n\pi} [\cos(\frac{n\pi}{2}) - 1] e^{-\lambda t} \sin(\frac{n\pi}{2l} x) \approx (T_0 - T_1) e^{-\lambda t}$$

$$\text{So } T_{top}(t) = T_1 + (T_0 - T_1) e^{-\left(\frac{\pi a}{2(L_1 + a\frac{L_2}{b})}\right)^2 t} \quad (9)$$

$$T_{bottom}(t) = T_1 + (T_0 - T_1) e^{-\left(\frac{\pi a}{2L_2\frac{a}{b}}\right)^2 t} \quad (10)$$

According to Equation (9), (10), the temperature difference between the top node and the bottom node of the sample layer becomes,

$$\Delta T = (T_1 - T_0) (e^{-\left(\frac{\pi b}{2L_2}\right)^2 t} - e^{-\left(\frac{\pi^2}{4L_2^2}\right) \left[\frac{L_1^2 b^4 + 2L_1 L_2 a b^3}{b^2 L_1^2 + L_2^2 a^2 + 2L_1 L_2 a b^3}\right] t}) \quad (11)$$

where T_0 is initial temperature of the heater, T_1 is final temperature of the heater, L_1 is the thickness of the sample layer, L_2 is the thickness of the glass layer, t is time.

For the sample layer, the thermal diffusivity can be expressed as $a^2 = \frac{K_1}{\sigma_1 \rho_1}$, where K_1 is the thermal conductivity, σ_1 is the specific heat, and ρ_1 is the density of material.

For the glass layer, the thermal diffusivity can be expressed as $b^2 = \frac{K_2}{\sigma_2 \rho_2}$, where K_2 is the thermal conductivity, σ_2 is the specific heat, and ρ_2 is the density of material.

Appendix E Choice of temperature sensing methods

Temperature sensor types can be simply classified into two groups, contact and non-contact. These two types of sensor require some assumptions and inferences to measure temperature. Many well-known uses of these sensors are straightforward and few, if any, assumptions are required. Other uses require some careful analysis to determine the controlling aspects of influencing factors that can make apparent temperature different from indicated temperature. Following sections will discuss on different types of temperature sensors that could be used in PCR microchip.

1 Contact sensor

Contact temperature sensors measure their own temperature. One infers the temperature of object to which the sensor is in contact by assuming or knowing that the two are in thermal equilibrium. Contact temperature sensors come in a variety of types and constructions.

1.1 Thermocouple

Thermocouples are among the easiest temperature sensors. They are based on the Seebeck effect that occurs in electrical conductors that experience a temperature gradient along their length. They are pairs of dissimilar metal wires joined at least at one end, which generate a net thermoelectric voltage between open pair according to the size of temperature difference between ends. Thermocouple will generate electricity when heat is applied to the tip of it. [62]

1.2 Resistance Temperature Detector

RTDs are among the most precise temperature sensors commercially used. They are based on the positive temperature coefficient of electrical resistance. RTD elements are wire windings or other thin film serpentines that exhibit changes in resistance with changes in temperature. Metals such as copper, nickel and nickel-iron are often used, however the most linear, repeatable and stable RTDs are constructed from platinum.

1.3 Thermistor

Thermistors abound inside many devices as well as in special temperature sensing probes. They typically work over a relatively small temperature range and can be very accurate and precise within that range. [62]

1.4 Thermochromic liquid crystal

Thermochromic Liquid Crystals (TLCs) are materials that change their molecular structure and optical properties with temperature [64]. In liquid crystals, the intermolecular forces in the crystalline solid are not the same in all directions. When this material is heated, the increased molecular motion overcomes the weaker forces first. Its molecules are still bounded by the stronger forces. This produces a molecular arrangement that is random in some directions and regular in others. [64]

2 Non-contact sensor

Most commercial and scientific non-contact temperature sensors measure the thermal radiant power of the infrared radiation that they receive and then infer the temperature of an object from which radiant power are assumed to be emitted. This type of non-contact sensor is commonly known as radiation thermometers. These thermometers measure temperature from the amount of thermal electromagnetic radiation received from a spot on the object of measurement. [62]

3 Selection of temperature sensors

Table 1: Temperature sensor comparison guide [62]

Temperature Sensor Attributes			
Criteria	Thermocouple	RTD	Thermistor
Cost-OEM Quality	Low	High	Low
Temperature Range	Very wide -450°F +4200°F	Wide -400°F +1200°F	Short to medium -100°F +500°F
Interchangeability	Good	Excellent	Poor to fair
Long-term Stability	Poor to fair	Good	Poor
Accuracy	Medium	High	Medium

Appendix E Choice of temperature sensing methods

Repeatability	Poor to fair	Excellent	Fair to good
Sensitivity (output)	Low	Medium	Very high
Response	Medium to fast	Medium	Medium to fast
Linearity	Fair	Good	Poor
Self Heating	No	Very low to low	High
Point (end) Sensitive	Excellent	Fair	Good
Lead Effect	High	Medium	Low
Size/Packaging	Small to large	Medium to small	Small to medium

Table 2: Advantages and disadvantages of temperature sensors [62]

Temperature Sensor Advantages/Disadvantages		
Sensor	Advantages	Disadvantages
Thermocouple	Self-powered Simple Rugged Inexpensive Wide variety Wide range	Non-linear Low voltage Reference required Least stable Least sensitive
RTD	Most stable Most accurate More linear than thermocouple	Current source required Low absolute resistance Self heating
Infrared	No contact required Very fast response time Good stability over time High repeatability No oxidation/corrosion to affect sensor	High initial cost More complex/support electronics Spot size restricts application Emissivity variations affect readings Accuracy affected by dust, smoke and background radiation

The selection of temperature sensors generally focuses on accuracy, response time, stability and repeatability. According to the above tables, RTD is most suitable for this chip design.

Appendix F Investigation of the effect of heater width and gaps on temperature uniformity

In the simulation chapter, it is assumed that the heater is located at the round area without any gaps, but actually the heater is a Pt thin film line deposited on the surface of the chip (shown as Figure 5.3). The width of the heater lines is $100\mu\text{m}$, while the gaps between them are $300\mu\text{m}$. To evaluate the effect of heater width and gaps on temperature uniformity during PCR, FEM simulations are conducted as following.

During the annealing stage, the heater would be turned off, thus there is no difference between the actual condition and assumption. Only the two heating stages need to be investigated.

Shown as Figure 1, to focus the concentration on the effect of heater width on temperature uniformity, only the middle part of the model is illustrated.

Figure 1: cross-sectional view of a PCR chamber model

In the denaturation stage, the temperature distribution is shown as Figure 2.

Appendix F Investigation of the effect of heater width and gaps on temperature uniformity

Figure 2: temperature distribution during denaturation. Assumption (up) and actual condition (down)

Shown as Figure 2, the temperature distribution in assumed condition is similar with that of the actual condition. In the sample part, the maximum temperature difference

Appendix F Investigation of the effect of heater width and gaps on temperature uniformity

between them is 0.241°C (located at the boundary of the top surface of the sample cylinder), which is only $1/8$ of 2°C temperature difference limit during rapid PCR.

In the extension stage, the temperature distribution is shown as Figure 3.

Figure 3: temperature distribution during extension. Assumption (up) and actual condition (down)

Shown as Figure 3, the temperature distributions in assumed condition and in actual condition are also similar. In the sample part, the maximum temperature difference between them is 0.475°C (located at the boundary of the top surface of the sample cylinder), which is also much less than 2°C temperature difference limit.

From above investigations, it is concluded that the Pt thin film heater deposited on the surface of the chip, with $100\mu\text{m}$ width and $300\mu\text{m}$ gap, can heat up the PCR sample with good temperature uniformity. Thus the assumption which regards the heat source as a uniform disk area is feasible.

Appendix G Resolution of PT-100 sensor using PT-104 Platinum resistance data logger [69]

PT-104 Platinum Resistance Data Logger

The PT-104 is a four channel temperature measuring data logger. It offers the ultimate in resolution (0.001 °C) and accuracy (0.01 °C) when used with PT100 sensors. It can also be used to measure resistance and voltage.

- Measures temperature, resistance and voltage
- High resolution and accuracy
- For use with PT100 and PT1000 sensors

A wide range of [PT-100 sensors](#) are available for use with the PT-104.

Accuracy and Resolution

Although accurate temperature sensors are widely available, it has been difficult to take advantage of them due to errors caused by the measuring device. The PT104 however, is inherently accurate due to its novel design. Rather than relying on voltage references (which tend to be temperature sensitive) it uses 'reference' resistors which are extremely stable (low temperature co-efficient and drift). The exact value of each resistor is stored in an EEPROM to provide the ultimate in accuracy (yearly re-calibration is recommended). To achieve the 0.001 °C resolution a state of the art ADC is used that can resolve to better than 1 part in 16 million.

Temperature

The PT104 measures temperature using platinum resistance thermometers (PRTs). Both common industry standards (PT100 and PT1000) are supported. The unit is compatible with 2, 3 and 4 wire sensors (4 wire PT100 sensors are recommended for accurate measurements). More information on sensors can be found below.

Resistance

When measuring resistance, the unit uses a four-wire circuit to give the greatest possible accuracy.

Two resistance ranges are available (0 to 375 Ohms and 0 to 10,000 Ohms). The unit is calibrated for 0 to 375 Ohms so this range should be used for accurate measurements.

Voltage

For voltage measurements, each input connector can be treated as a differential input with ground, or two single ended inputs. Both inputs must be zero Volts or above, though it does not matter which input has the higher voltage.

Two voltage ranges are available (0 to 115 mV and 0 to 2500 mV). For accurate measurements use the 0 to 2500 mV range.

PT-104 Specifications

	Temperature	Resistance	Voltage
Sensor	PT100*, PT1000	N/A	N/A
Range	-200 to 800 °C	0 to 375 Ω* 0 to 10 kΩ	0 to 115 mV 0 to 2.5 V*
Linearity	10 ppm	10 ppm	10 ppm
Accuracy @25 °C	0.01 °C*	20 ppm*	0.2%*
Temperature coefficient	3 ppm/°C	3 ppm/°C	100 ppm/°C
RMS Noise (using filter)	0.01 °C	10 ppm	10 ppm
Resolution	0.001 °C	1 μΩ	0.156 μV
Conversion time per channel	720 mS **	720 mS **	180 mS
Number of inputs	4		
Connectors	4-pin miniDIN		
Input impedance	>>1 MΩ		
Overvoltage protection	±100 V		
Output	RS232, D9 female		
Environmental	20 to 30 °C for stated accuracy, 0 to 70 °C overall, 20 to 90% RH		