Development of IR-Mediated Thermocycling System And Multi-Reaction PCR Chip For DNA Amplification

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ABSTRACT

The applications and development of polymerase chain reaction (PCR) for DNA analysis is becoming important in molecular diagnostics and drug development. Many conventional PCR systems use glass or plastic based tubes with relatively big volumes (>25 µL) to conduct PCR. Thus, this resulted in more reagents consumed and longer reaction time to reach thermal balance because their larger volume tubes and slow thermal cycling. With the applications of MEMS technologies, PCR microchip can be realised. These PCR microdevices can greatly reduce the DNA analysis cost with less consumption of reagent and offers the potential of rapid thermal cycling.

The present work is aimed to design, analyse and fabricate a multi-reaction PCR chip and PCR thermocycler for DNA amplification. The chip will consist of multiple reaction vessels for high throughput PCR. The thermocycler will perform heating processes by infra-red (IR) radiation, and cooling process by forced convection. In addition, the thermocycler is capable of on-board real-time PCR products detection.

The objective of the present work is to improve the PCR amplification process with faster heat transfer rates and better temperature uniformity. 3-D transient numerical simulations using FEMAP TMG software and ICEPAK 4.0 were conducted to assess and verify the performance of the proposed design. The results indicated that faster PCR thermal cycling with better temperature uniformity could be achieved in this PCR chip and thermocycler design.

A prototype of the PCR chip consists of an array of capillaries as reaction chambers for the PCR mixture. Capillaries are widely used for molecular research and are readily available in large quantities. Hence, the chip fabrication processes are simple and inexpensive, as no costly and time-consuming techniques are required. Each capillary has a volume of 7 µl and fabricated on a substrate base. The prototypes of the PCR chip and thermocycler are tested experimentally for their feasibility and performance. A PCR process of 40 cycles is conducted using a standard protocol for the PCR mix. The linear
image sensor of the PCR thermocycler is able to detect the amplified PCR products successfully. The PCR chip and thermocycler is found to be capable of performing PCR successfully with real-time fluorescence detection.
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Chapter 1: INTRODUCTION

1.1 Background

The December of the year 2003 marks the 18th anniversary of a paper that significantly changed biological research. On December 20, 1985, Randall Saiki et al. published their paper entitled, "Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia" [1] in the journal Science. In this paper, Saiki et al. [1] described a method to detect single base pair changes in the beta-globin gene that result in sickle cell anemia and became the first to use the polymerase chain reaction (PCR). This is an important step forward in modern molecular biology. But the method as originally described was time-consuming and labor intensive. 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. This is caused by an increase in the fluorescence of EtBr when in the presence of double-stranded DNA (dsDNA). This fact was further exploited in 1991, when Higuchi et al. [4] introduced the kinetic PCR (also known as real-time PCR), which allows real time monitoring of the DNA amplification process. This approach also provides a means of determining the effect of different reaction conditions on the efficacy of the amplification, thus provides an insight into fundamental PCR processes.

In a 1987 Methods in Enzymology publication, Mullis and Faloona [5] described the process of PCR in detail: "the solution (PCR reaction) is brought to 100°C for 1 min, and is cooled to 25°C for 30 sec in a water-bath." Fortunately, for those who use PCR, it’s no longer necessary to coordinate 3 water-baths at the correct temperatures. Technology has stepped in, and brings about the development of the PCR thermocycler.

All of these developments have helped lead to a simpler and more efficient PCR and improvements to the process are continued. Today, the use of polymerase
chain reaction (PCR) in molecular diagnostics has increased to the point where it is accepted as the gold standard for detecting nucleic acids from a number of origins and it has become an essential tool in the research laboratory [82]. It is based simply on the unique biochemistry of DNA replication and allows researchers to "amplify" specific DNA sequences from samples that contain only a few individual DNA molecules with sequences complementary to the primers.

In the Human Genome project, an effort is being made to accumulate genetic sequence data by many organizations, where hundreds of PCR instruments are used continuously. The efficiency and cost of PCR has therefore become an important design criterion [6]. The wide spread applications of PCR has also resulted in the development of many devices or systems to provide the thermal cycling necessary for PCR. These devices are based on a variety of design principles for heat transfer, including water baths, air baths, dry blocks and micro-devices fabricated from silicon. Widely different conditions are implemented to achieve amplification. Conditions such as cycling times, operating temperatures and reactant volumes and composition varied between the different systems.

In recent years, the PCR technique 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. Highly integrated hybridization assay and capillary electrophoresis have been reported which improve the throughput of DNA analysis. This shift to high throughput analysis requires a high speed DNA amplification system, and rapid PCR systems have been developed with this purpose in mind. Therefore, the overall aim of these developments has been to reduce cost, time of reaction and contamination, while at the same time, achieving adequate amplification of the targeted nucleotide sequence for subsequent detection or analysis [7].

Enormous amount of data generated by conventional PCR assays has provided the foundation of real-time PCR. Real-time PCR allows the monitoring of accumulating PCR products in real-time, which has been made possible by the labeling of primers, probes or amplicon with fluorogenic molecules. It has proven
itself valuable in laboratories around the globe and has distinct advantages over radiogenic oligoprobes commonly used in conventional PCR assays. These benefits include an avoidance of radioactive emissions, ease of disposal and an extended shelf life [82].

1.2 Objectives

Current PCR chip and thermocycler development has reported that PCR by infrared radiation heating could be performed on a single chamber PCR microchip. Therefore, the motivation for this project is to design a PCR thermocycler that is capable of infra-red (IR) radiation heating on a PCR chip of multiple reaction chambers. In addition, the proposed designs would ensure that efficient temperature ramping and thermal uniformity could be attained.

In the other words, the aim of this report is to design and develop a PCR system with the following features:

- Rapid thermal cycling by utilizing infrared radiation heating and forced convection cooling
- Good temperature uniformity achieved for the PCR reaction chambers
- Disposable or low cost multi-chamber PCR reaction chip design

1.3 Scope

Chapter One, the introduction of this report, describes the motivation for the project. The objectives are also stated.

Chapter Two explains the working principle of Polymerase Chain Reaction (PCR) process. This is followed by a review of conventional PCR thermocyclers and their limitations. In addition, research works in the area of PCR miniaturization are also reviewed.

Chapter Three presents the development of the PCR thermocycler and PCR chip designs. These are largely based on investigations of a preferred heat transfer
mechanisms, the selection of an adequate material for the PCR chip and an accurate temperature sensing device that could be incorporated into the PCR system designed. The primary concern is to be able to achieve rapid PCR thermal cycling and maintaining uniform temperature within the PCR sample.

Chapter Four consists of an extensive finite element analysis on the proposed PCR thermocycler and PCR chip designs during the heating processes, namely denaturation and extension processes. From the analysis of results on the rate of heating and thermal uniformity, the thermocycler and chip designs are remodified.

Chapter Five investigates on the cooling rate and thermal uniformity using the CFD analysis software. This assesses the performance of the PCR thermocycler and chip design during annealing process. Similarly, improvements on thermocycler and chip design are made based on the analysis results.

Chapter Six explains the fabrication of the PCR chip design. The experimental tests that were carried out are presented as well. This is followed by a discussion of the experimental results.

Chapter Seven is the conclusion summarizing the project work that has been done. Recommendations for future work are proposed to improve the PCR thermocycler and chip design.
Chapter 2  

LITERATURE REVIEW

2.1 Introduction to DNA

Genetic information of humans is stored in the cell chromosomes. Each chromosome consists of long, compactly packed and super coiled linear polymer strands of deoxyribonucleic acid (or DNA). The chromosome information is stored as a long string of DNA fragments grouped as genes, each expressing an identifiable function or characteristic of the organism [9].

2.1.1 Chemical structure of DNA

The units of a single DNA strand are called nucleotides. Each nucleotide consists of a base (B), a sugar linkage (S), and a phosphate bridge (P), as seen 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) through weak hydrogen bonds to another strand of complementary base pairs (G-C and A-T) forming 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 [9].

![Figure 2.1: (a) DNA molecule, (b) single stranded DNA chain, and (c) double stranded DNA with complementary base pair (bp). The two strands are joined by hydrogen bonds [34].](image-url)
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This base pairing characteristics of the nucleotides are responsible for the pairing of two chains to form a strongly stabilized duplex, as seen in Figure 2.1 (c). When two chains align themselves in this way, they assume a double-helical structure [104]. All information relevant to cell growth and regulation is stored in this form. To perform an analysis, first the DNA must be extracted from the cell nucleus and purified. This procedure involves the disruption of cell membranes by chemical exposure to a detergent. This is typically followed by purification using centrifugation or other methods for removal of cell debris leaving DNA in solution [9].

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 the 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.

2.1.2 Size of DNA

DNA is long and unbranched [104]. A one-foot long string or strand of DNA is normally packed into a space roughly equal to a cube 1/millionth of an inch on a side. This is possible only because DNA is a very thin string. DNA is commonly sized in terms of base pairs. (e.g. 100bp, 1kp = 1000bp) This is measured using DNA size markers. An example of a DNA marker is given below:

\[ \text{e.g. DNA sample moves through agarose gel by electrophoresis.} \]

This gel acts as a sieve for the DNA separation according to size. Bigger DNA molecules move slower while smaller DNA molecules move faster within the gel. Migration distance from the start point should be about the same for molecules of same size. Thus, different sizes of molecules migrate at different distances. The end result is shown below (Figure 2.2):
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Figure 2.2: DNA size markers [73]

Figure 2.3: size of DNA [74]
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Length in "angstroms," a unit of measure is equal to 1 hundred-millionth of a centimetre. That means that 1 angstrom is equal to $1 \times 10^{-10} \text{m}$.

Bustamante et. al. [23] had investigated two-dimensional diffusion of DNA on mica. Scanning force microscopy, operating in liquids, were used to directly visualise the movements of *E. coli* RNA polymerase on non-specific DNA, thus providing direct evidence for the mechanisms of facilitated target location. Images were acquired on DNA being deposited on a mica surface, where they interact with *E. coli* RNA polymerase present in the solution covering the surface. The DNA molecules used in their experiment has 1047 bp and these DNA molecules are found to occupy a two-dimensional space of approximately 254.54 nm by 145.45 nm.

To achieve DNA fragments of about 1 kp, restriction digestion would be used. This process uses an enzyme known as restriction enzymes (RE) to segment the DNA strand to the required base-pairs. The process, in short, requires the RE to mix with the DNA strand and incubated at a specific temperature for a specific time. Both the incubation temperature and time is dependent on the RE used.

2.2 PCR polymerase

DNA samples are often present at concentration levels that are too low for any direct tests. Several schemes can be used for DNA amplification but all of these require powerful enzymes. Enzymes are "miracle workers" protein catalyst molecules that can manipulate and modify DNA strands present in every living organism [9]. In particular, enzymes assemble complementary strands of DNA from a single strand fragment. This enzyme scans single strands of DNA and starting from a specific location, captures matching nucleotides from the PCR mixture, and connects them to the single strand, assembling the complementary strand one base at a time.
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The choice of the correct enzyme(s) to use in the PCR process is determined by several factors. Taq DNA polymerase, the first enzyme used for PCR, is still the most popular and is the basis for nearly all PCR thermostable enzymes. Any modifications are often used to improve its specificity e.g. Hot-start polymerase, AmpliTaq Gold polymerase, Platinum Taq polymerase and Titanium Taq polymerase. If polymerase is not thermostable, new enzymes must be added during each thermal cycle, which will result in a tedious and error prone process [2]. Nowadays, almost all PCR reactions use thermostable enzymes.

Taq polymerase possesses relatively high processivity and is the least expensive choice. It generates PCR products with single deoxyadenosine overhangs on the 3'-ends. These overhangs allow easy cloning into "T"-vectors (for example, Promega's pGEM®-T, pGEM®-T Easy and pTATGE™ Vectors), which possess "T" overhangs complementary to those on the PCR product. Enzymes, which lack 3'-->5' exonuclease ("proofreading") activity are sometimes recommended to ensure accurate amplification of the PCR product. These enzymes (such as Tli DNA Polymerase) normally generate blunt-ended PCR products.

2.2.1 **Size of PCR polymerase**

Since the enzymes are protein molecules, proteins and other bio-molecules like DNA and cell membranes, have a size of several nanometres. For example, E. coli RNA polymerase, a type of enzyme, is found to occupy a two-dimensional space of approximately 36.36 nm by 18.18 nm [23].

2.3 **Polymerase chain reaction**

Polymerase Chain Reaction (PCR) is an uncomplicated technique. All that happens is that a short region of a DNA molecule is copied many times by a DNA polymerase enzyme. This may seem trivial but it has a multitude of applications in genetical research and in broader areas of biology.
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PCR results in the selective amplification of a chosen region of a DNA molecule. Any region of a DNA molecule can be chosen, as long as the sequences at the borders of the region are known. The border sequences must be known to allow two short oligonucleotides to hybridise to the DNA molecule, one to each strand of the double helix (see Figure 2.4). These oligonucleotides are known as *primers* that delimit the region that will be amplified.

![Figure 2.4](image.png)

Figure 2.4: Hybridization of the oligonucleotide primers to the template DNA at the beginning of PCR [8]

Thereafter, amplification is carried out by the DNA polymerase enzymes, which are extracted from a heat-resistant micro-organism (*Thermus aquaticus*). These enzymes, including the Taq polymerase, are thermostable, which mean that they are resistant to denaturation by heat treatment [38]. The thermostability of polymerase is an essential requirement in PCR methodology.

The chemistry of PCR depends on the complementarity of the DNA bases [78]. To begin amplification, the polymerase is added to the primed template DNA and
incubated, so that it synthesizes new complementary strands (see Figure 2.5a). The mixture is then heated to 94°C, so that the newly synthesized strands detach from the template (as shown in Figure 2.5b), 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 2.5c). 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 2.5d.

![Figure 2.5: PCR reaction [8]](image)

At the end of the PCR experiment, a sample of the reaction mix is usually analysed by agarose gel electrophoresis. Sufficient DNA has been produced for the amplified fragment to be visible as a discrete band after staining with ethidium bromide (Figure 2.5e).
2.3.1 Oligonucleotide primers for PCR

Primers are important to the success or failure of a PCR experiment. If the primers are designed correctly, the experiment will result in specific amplification of a single DNA fragment, corresponding to the target region of the template molecule. If the primers are incorrectly designed, then the experiment will fail, possibly because no amplification occurs, or possibly because the wrong fragment, or more than one fragment, is amplified. Clearly, the primers must correspond with the sequences bordering the target region on the template molecule. Each primer must be complementary (not identical) to its template strand in order for hybridisation to occur, and the 3’ ends of the hybridised primers should point towards one another (Figure 2.6). The DNA fragment to be amplified should not be greater than about 3 kb in length and ideally will be less than 1 kb. Fragments up to 10 kb can be amplified by standard PCR techniques, but the longer the fragment, the less efficient the amplification and the more difficult it is to obtain consistent results. Amplification of very long fragments up to 40 kb is possible but requires special methods [8, 78].

![Diagram of human α1-globin gene](image)

Figure 2.6: A pair of primers designed to amplify the human α1-globin gene [8]

Similarly, the length of the primers will influence the rate at which it hybridises to the template DNA. A primer, which is too short, might hybridise to non-target
sites and give undesired amplification products. Conversely, longer primers hybridise at a slower rate, thus the efficiency of PCR, measured by the number of amplified molecules produced during the experiment, is therefore reduced if the primers are too long, as complete hybridisation to the template molecules can not occur in the time allowed during the reaction cycle [8].

### 2.3.2 Temperatures of a PCR cycle

The theoretical DNA amplification factor is $2^n$, where $n$ defines the number of PCR cycles performed [11]. In each cycle of a PCR, the reaction mixture is experiencing three different temperatures (Figure 2.7) [78]:

1. The denaturation temperature, usually about 90 to 96°C, breaks the base pairs and releases single-stranded DNA, which will act as templates in the next round of DNA synthesis.
2. The hybridisation or annealing temperature, usually about 40 to 60°C, is the temperature at which the primers attach to the templates.

![Figure 2.7: A typical temperature profile of a PCR](image)
3. The extension temperature causes DNA synthesis occurs. This is usually set at about 70 to 75°C, just below the optimum for Taq polymerase.

The stated temperature is just generalization and that optimum temperatures or temperature ranges for each stage of the PCR reaction must be determined based upon the particular reaction being run. These optimal temperatures will depend on factors such as the particular DNA being replicated, the number of cytosine-guanine hydrogen bonds versus the number of adenosine-thymine bonds, the enzyme being used, the length of the primer pair. Precise maintenance of these optimum temperatures can effect the reaction in numerous ways. For example, while the temperature at which DNA denatures is generally about 90 and 96°C, 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 [8, 10].

During the PCR cycle, achieving the annealing temperature is most important as it affects the specificity of the reaction. DNA-DNA hybridisation is a temperature dependent phenomenon. If the temperature is too high, then no hybridisation takes place, instead the primers and templates remain dissociated (Figure 2.8a), resulting 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 (Figure 2.8b). If these mismatches are tolerated, then the number of potential hybridisation sites for each primer is greatly increased, and amplification is more likely to occur at non-target sites on the template molecule, thus resulting in non-specific DNA fragments to be amplified. The ideal annealing temperature must be low enough to enable hybridisation between primer and template but high enough to prevent mismatched hybrids from forming (Figure 2.8c). This temperature can be estimated by determining the melting temperature of the primer-template hybrid [8, 10, 38]. The annealing temperature should be 1-2°C below the melting temperature, which is low enough to allow the correct
primer-template hybrid to form, but too high for a hybrid with a single mismatch to be stable [8].

Figure 2.8: Temperature has an important effect on the hybridisation of primers to template DNA [8]

Attaining the optimal temperature for the extension reaction is also important for obtaining the desired PCR result. Temperature may affect both the rate and accuracy of the extension reaction. If the rate of the polymerase reaction is too slow, then the newly synthesized polynucleotide may not contain a site for primer annealing. Additionally, the denatured polynucleotide sequence for amplification may contain one or more regions of secondary structure that may form or disappear according to the temperature selected. Furthermore, several different enzymes with polymerase activity may be used for PCR. Each enzyme will have its own optimum temperature for activity, stability and accuracy [8, 10].

Therefore, the determination of optimum temperatures for PCR is important to ensure its success. In addition, any unnecessary temperature elevation, such as
those experienced with slow temperature transitions, should be avoided. This inability to perform rapid and sharp transitions between temperatures also causes inaccurate PCR results. For example, a lag time between the denaturation temperature and the annealing temperature can result in unspecific annealing. Long transition times between denaturation and annealing also promote the premature inactivation of the polymerase enzyme, resulting in a less specific reaction and in lower product yield [8].

2.3.3 Analysis of PCR products

The amplification product is studied in various ways in order to gain information about the DNA molecule that acted as the original template. The three most important techniques to study PCR are as follows [8]:
1. Direct detection by ethidium bromide
2. Detection by gel electrophoresis
3. Blotting and hybridisation with specific probes
4. Detection by a combination of capture and detection probes
5. Sequencing of the PCR product
6. Labelling of oligonucleotide probes and primers

2.3.3.1 Direct detection of ethidium bromide

The simplest method to detect a PCR product is to add a small quantity of ethidium bromide, a dye that becomes fluorescent after intercalating with strands of DNA (see Figure 2.9), to the reaction vessel after the amplification procedure.
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Figure 2.9 (a) DNA nucleotide, (b) single-stranded DNA chain and (c) double stranded DNA with complementary base pair. The two strands are joined by hydrogen bonds [9]

When the reaction vessels are examined under an ultraviolet lamp, a positive reaction will emit light whereas negative reactions remain dark [82, 78]. A single DNA molecule can contain hundreds of fluorophores and emit a strong signal. These light emitting DNA fragments can be observed using an epi-fluorescence microscopes [9].

2.3.3.2 Detection by gel electrophoresis

The results are checked by running a portion of the amplified reaction mixture in an agarose gel. A band representing the amplified DNA may be visible after ethidium bromide staining (Figure 2.10). Ethidium bromide is a type of intercalating dyes which fluorescence when excited by UV light only when bound between two nucleotides in double-stranded DNA [78]. Figure 2.10 shows a fragment accommodating one intercalating label per
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Figure 2.10: Gel electrophoresis of the PCR product can provide information on the template DNA molecule [8]

In Figure 2.10, Lanes 1 and 2 show, respectively, an unrestricted PCR product and a product restricted with the enzyme that cuts at site 'R'. Lane 3 shows the result obtained when the template DNA contains an insertion in the amplified region.

2.3.3.3 Blotting and hybridisation with specific probes

When screening for known point mutations in the sequence amplified by PCR, amplicons can be transferred (blotted) to nitrocellulose or nylon membranes and analysed by hybridisation with a labelled oligonucleotide probe specific for the mutation. Washing the membrane at a critical temperature after hybridisation removes probes bound to sequences that were not a perfect match [78]. However, this is a time-consuming process, as it requires multiple PCR product handling steps, thus further increasing the risk of contamination [82].

2.3.3.4 Detection by a combination of capture and detection probes

The increased automation of PCR and the detection of PCR product have led to the development of more-sophisticated techniques to detect whether
specific amplification has taken place during the reaction. As an alternative to analysis by electrophoresis and blotting procedures, the PCR product can be detected by a combination of capture and detection probes. In addition to the two primers used for amplification, two oligomers are designed to hybridise as probes within the amplicon. One probe is used for capture (that is, for the binding of the PCR product to a solid phase such as micro plate, membrane or paramagnetic particle) and the other is labelled and used to detect the immobilized PCR product [78].

2.3.3.5 Sequencing of the PCR product

The nucleotide sequence of the PCR product can be determined by sequencing either directly or after cloning into an appropriate vector. Sequencing the amplified stretch of DNA can be particularly important in the case of infection with HIV or hepatitis C virus (HCV) in order to determine the extent of sequence variability in regions within the envelope genes [78].

The sequencing method requires single-stranded DNA as the starting material. The single-stranded DNA undergoes a procedure known as Sanger-Coulson method, in which a DNA polymerase synthesizes families of chain-terminated molecules. This reaction thus generates complementary strand fragments terminated at all possible positions of the matching dideoxide. These fragments can be separated in a gel and their relative positions indicate the location in the sequence where a specific base is present, hence the sequence could be read out directly [8].

23.3.6 Labelling of oligonucleotide probes and primers

Although PCR products can be directly visualized by staining with ethidium bromide, a higher degree of sensitivity in the detection of PCR product can be achieved by labelling one of the PCR primers or by hybridisation with a labelled oligonucleotide probe. Common examples are fluorescent labels that can be directly detected by means of an
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automated gel detection system or by microscopy (as discussed in section 2.3.3.1). Another example of detection systems is based on linking an enzyme to the oligonucleotide. The enzyme catalyses a reaction that produces a coloured product. These enzymes can directly cross-link to the oligonucleotide. Thereafter, enzymatic detection takes place [78].

As illustrated here, a typical DNA assay may involve one or a combination of the above techniques. For example, the process may include the extraction of nucleic material from a cell followed by amplification, staining, restriction digestion, and electrophoretic separation.

2.3.4 Real-Time Quantitative PCR

Theoretically, as stated in section 2.3.2, the theoretical DNA amplification factor is $2^n$ at any given cycle. However, in practice, the molecular amplification process is not that straightforward. First, it is important to understand what happens during an actual PCR reaction. A basic PCR run can be broken up into three phases (see Figure 2.11) [90]:

- **Exponential**: Exact doubling of product is accumulating at every cycle (assuming 100% reaction efficiency). The reaction is very specific and precise.
- **Linear (High Variability)**: The reaction components are being consumed, the reaction is slowing, and products are starting to degrade.
- **Plateau (End-Point: Gel detection for traditional methods)**: The reaction has stopped, no more products are being made and if left long enough, the PCR products will begin to degrade.
Initially, exponential occurs because all of the reagents are fresh and available, and the kinetics of PCR pushes the reaction to favor doubling of amplified products. As the reaction progresses, some of the reagents are being consumed as a result of the amplification. This depletion will occur at different rates for each replicate. The reactions start to slow down and the PCR product is no longer being doubled at each cycle. This linear amplification can be seen in the linear phase of the reaction. The amplification eventually slows down and reaches a plateau. This plateau phase is also where traditional PCR takes its measurement and is known as end-point detection. In addition, the amplification may not be exponential to begin with because of variations in reaction conditions or the presence of inhibitors. Hence, end-point detection might cause the number of copies of molecular amplification to be inaccurate due to variable yield at the plateau phase (see Figure 2.12).
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On the other hand, real-time PCR measures the data at the exponential phase of the PCR reaction, thus allowing amplification measurements to be recorded as the reaction occurs. This eliminates the variability traditionally associated with quantitative PCR, and allows routine and reliable quantitation of PCR products [12]. Likewise, this also means that laborious post PCR methods are not required, thus saving time and costs. In comparison to traditional PCR procedures that required electrophoresis to determine the presence of products, real time PCR are found to have increased sensitivity, specificity and rapidity of the assay [38].

There are two widely used methods to perform real-time PCR analysis namely:

- 5’ Nuclease assay
- SYBER Green Dye Assay

### 2.3.4.1 5’ Nuclease assay

5’ Nuclease assay is a kinetic PCR protocol that utilizes the AmpiTaq Gold®DNA Polymerase. This polymerase has 5’ exo-nuclease activity (see Figure 2.13) and together with FRET (Fluorescent Resonant Energy Transfer), it is possible to detect PCR amplification in real-time.
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The 5' exo-nuclease activity of the enzyme acts upon the surface of the DNA template to remove obstacles downstream of the growing amplified product that may interfere with its generation. The 5’ nuclease assay uses this activity in real-time detection.

In Figure 2.14 below, the polymerase adds bases to the growing chain of DNA. Subsequently, the polymerase removes DNA that is downstream, hence impeding its’ capability to synthesize the new strand.

In the 5’ nuclease assay, an oligonucleotide called a TaqMan®Probe is added to the PCR master mix. The probe is designed to anneal to a specific sequence of template between the forward and reverse primers. The probe sits in the path of the enzyme as it starts to copy DNA. When the enzyme reaches the annealed probe, the 5' exonuclease activity of the enzyme cleaves the probe, Figure 2.15 through 2.17.
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![Figure 2.15: The 5' Nuclease Assay [90]](image1)

![Figure 2.16: Polymerase collides with TaqMan® Probe [90]](image2)

![Figure 2.17: Cleavage of the TaqMan® Probe [90]](image3)

The principle of FRET is that when a high-energy dye is in close proximity to a low-energy dye, there will be a transfer of energy from high to low. In this case, the TaqMan® Probe is designed with a high-energy dye termed a Reporter at the 5' end, and a low-energy molecule termed a
Quencher at the 3’ end. When this probe is intact and excited by a light source, the Reporter dye's emission is suppressed by the Quencher dye as a result of the close proximity of the dyes, Figure 2.18. When the probe is cleaved by the 5’ nuclease activity of the enzyme, the distance between the Reporter and the Quencher increases and causes the transfer of energy to stop. The fluorescent emissions of the reporter increase and the quencher decrease, as seen in Figure 2.18.

![Figure 2.18: Increased fluorescence activity due to the cleaved probe [90]](image)

During the extension phase of PCR, the probe will anneal downstream from one of the primer sites and is cleaved by the 5' nuclease activity of the Taq DNA polymerase as this primer extends. Hence, this results in the increase of the reporter signal. Cleavage will also displace the probe from the target strand, allowing the primer to extend to the end of the template strand. The probe inclusion does not inhibit the overall PCR process. Reporter dye molecules are cleaved from their respective probes with each cycle, resulting in an increase in the fluorescence intensity proportional to the amount of amplification produced (Figure 2.19)[13].
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The combination of FRET and the 5' nuclease activity of AmpliTaq Gold® DNA Polymerase enables the 5'nuclease assay and the SDS instrumentation to collect data in real time.

2.3.4.2 SYBR Green Dye Assay

SYBR Green chemistry is an alternate method used to perform real-time PCR analysis. SYBR Green is a dye that binds the Minor Groove of double stranded DNA. When SYBR Green dye binds to double stranded DNA, the intensity of the fluorescent emission increases. As more double stranded amplified products are produced, SYBR Green dye signal will increase. Figures 2.20 show the entire process of each type of real-time chemistry. SYBR Green dye will bind to any double stranded DNA molecule, while the 5' nuclease assay is specific to a pre-determined target.
From Figure 2.20, we see that SYBR dye attaches to the double-stranded DNA. As the DNA template is amplified, more SYBR Green dye is attached and thus, emits a strong fluorescence signal. Monitoring this fluorescence increase allows real-time DNA detection.

Most fluorescent dyes are heterocyclic or polyaromatic hydrocarbons. That means light is absorbed by the dye, which leads to an excited state of the fluorophore that has a lifetime of $10^{-9} - 10^{-8}$ seconds (nanoseconds). They absorb and emit in the visible or near infrared regions. When the fluorophore returns from the first excited state to the ground state, light is emitted at lower energy (longer wavelength) than was originally captured.
The wavelength difference between the light captured (absorbance/excitation) and released (emission) is called the Stokes shift. A large Stokes shift is desirable as it simplifies the technical aspects of achieving concurrent excitation with emission detection and also decreased interfering background.

2.4 Review of Conventional PCR Devices

Conventionally, the standard PCR-based DNA assay is performed with a programmable thermocycler, that heat and cool a tube, capillary or slide containing the DNA sample mixture. The PCR amplification product is then delivered to a separate detection device such as the fluorescence analyser or the gel electrophoresis analyser for measurements. Most of the available PCR thermocyclers are no longer the large desktop devices it used to be. Today, there are many thermocyclers available on the market, all with features that are designed to make PCR faster and more accurate.

An example of a modern conventional PCR thermocycler is the Primus Thermal Cycler, from MWG Biotech, which is equipped with the High Temperature Range (HTR) Peltier technology. Phenomenal performance is the result: heating rates up to $4^\circ$C/sec, unique block homogeneity ($\pm 0.5^\circ$C) and extreme block durability to provide reliable results and highest reproducibility.

2.4.1 Disadvantages of Conventional PCR Devices

There are some common restrictions associated with conventional PCR devices. These include:

1. Slow thermal cycling and poor thermal uniformity

The basis of most commercial products for heating a sample is through intimate contact between a PCR reaction chamber holding the sample and a heating block that is rapidly heated and cooled (for example, by using a Peltier element that can both heat and cool) [10]. This heating/cooling block has a large heat capacity, which limits the rate at which the samples are heated and cooled [17, 18]. The
conventional PCR devices were capable of achieving up to 4°C/s in the temperature range of 50-94°C and required more than an hour to run a complete PCR reaction. Thus, with pre-PCR processing and PCR detection, the whole process can take several hours and at a cost of several hundred dollars [18, 19, 34, 37]. In addition, conventional systems require relatively high reaction volume (>25 µL), thus contributing to a slower thermal cycle [37, 63]. Moreover, in practice, overheating and sub-cooling cannot be entirely avoided. Furthermore, one of the greatest problems with PCR carried out in plastic PCR plates (e.g. 96 well-plates) are that the temperature gradients within the well plate maybe as high as 10°C. Therefore, there is a need to improve the performance of these PCR devices.

2. High power consumption and large size

The high power consumption and size of the conventional PCR thermocyclers are two other restrictions of preventing these devices from becoming portable for field use.

3. High risk of contamination

The products of the PCR is to be delivered with pipettes into the detection devices, thus there is a lot of sample handling activities. This increases the risk of contamination of PCR products by the surrounding environment, and subsequently affecting the accuracy of results of the DNA assays.

2.5 PCR chip developments and miniaturization

Miniaturization concepts have recently been brought to the forefront of the analytical sciences. 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 equipments [6]. In recent years, many devices for PCR have been constructed to demonstrate the benefits of miniaturizing. These devices are commonly referred as 'PCR chips'. This micro-PCR device, based on micro-electro-mechanical systems (MEMS) technology,
provides a platform for PCR amplification and in situ fluorescence detection, while others try to accommodate all the necessary fluid, thermal and detection functions under a common substrate to realize the conception of the miniaturized total chemical analysis system (µTAS) [21, 40]. These lead to the development of high performance and reusable µPCR devices. With the possibility of low cost fabrication, disposability of these devices is also investigated.

2.5.1 Review of the development of PCR chips and thermocyclers

A number of micro-devices have been developed to accomplish faster PCR thermal cycles by basically reducing or eliminating the large thermal mass present in macroscopic systems. One of the earlier device developed is from Poser et al [19]. They developed chips with one, two (as seen in Figure 2.21) and ten chambers and volumes of reaction chambers in the range of 5 to 10 µL to investigate into the feasibility of miniaturizing thermocyclers.

![Figure 2.21: Sketch of two-chamber chip. 1. inlet; 2. cover; 3. adjustment; 4. reaction chamber; 5. air chamber; 6. thin film heater; 7. temperature sensor; a. cover; b. topside; c. backside [19].](image-url)
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A unique feature of the design in Figure 2.22 is the inclusion of the air chamber, which insulates the reaction chamber to prevent heat loss by conduction to the surrounding material, thus allowing a more uniform temperature distribution within the reaction chamber. From results obtained, Poser et al. concluded that miniaturized thermocycler has several advantages, namely very short heating and cooling rates and consequently short cycle times can be achieved, thus resulting in a new potentiality for fast PCR. Miniaturized thermocyclers with locally optimized heat flow are also found to be a very efficient tool for precise management of reaction conditions in fast PCR processes.

On the other hand, Kalinina et al. [22] monitored PCR in volumes of the order of 10 nl in glass microcapillaries (as seen in Figure 2.22) using fluorescence energy transfer assay (TaqMan assay). From their investigations, when the PCR volume was reduced to 10 nl, a single target molecule was able to generate sufficient saturating concentration of PCR product after ~30 cycles. The PCR products could be detected using the TaqMan fluorescence energy transfer system.

Wilding et al. [7] has also developed an early model of PCR chip with photolithographed silicon. The PCR reaction chamber (sample volume of < 10µL) is produced using standard photolithographic procedures, as shown in Figure 2.23 below.
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Figure 2.23: Single chamber PCR chip etched to a depth of 80 µm [7].

The PCR chip [7] is held in a custom-fabricated device and located on a thermoelectric heater-cooler assembly, seen in Figure 2.24.

Figure 2.24: Schematic of holder for silicon PCR chip incorporating a heater-cooler assembly and luer fittings to which syringes would attach for removal of samples [7].

The thermal characteristics of silicon, coupled with the high surface to volume ratios are particularly advantageous features of this micro-machined device. One complete cycle of PCR takes 3 minutes and evidence of successful amplification of specific DNA sequences are demonstrated by conventional electrophoresis.

Another type of PCR chip is a continuous flow PCR device developed by Kopp et al. [11]. This device has a channel on three differential temperature zones, where
the temperatures are constantly maintained. The sample was processed across each temperature zone in turn (see Figure 2.25).

![Figure 2.25: Layout of chip [11]](image)

Hence, the thermal variation of heating blocks is unnecessary in this device. The pattern of the chip layout determines the relative time a fluid element is exposed to each temperature zone. This is an excellent approach as the heating and cooling rates are directly affected only by the heat capacity of the sample solution. It is not affected by the heating/cooling step of the chamber component and the heating/cooling block, and thus it is a very fast process. 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. [11], Nagai et al. [17] fabricated a silicon based PCR chamber array by semiconductor technology. This device consists of numerous miniaturized PCR chambers (see Figure 2.26 below), whereby each chamber is of pico-liter quantity. This reduces the heat capacity of the PCR sample. On average, the pico-liter chamber will contain on average 1 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 the other template [17].
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Figure 2.26: SEM images of 80 x 80 µm microchambers [17].

Three hotplates, constantly controlled at 95, 55 and 72°C, are utilized. Thus, the large thermal mass that is commonly associated with the heating/cooling block is eliminated. The device is repeatedly moved on each hotplate. This heating and cooling process is found to be capable of attaining a maximum rate of approximately 16°C/s. Throughput could be enormous with the integration of more microchambers.

Furthermore, Nagai et al. [6] designed four different types of micro-chambers of pico-liter quantity. The aim is to optimise fluid retention, thus the surface states of the substrate and the inner walls were examined. The retaining ability of the solution was examined by the following procedure: the water was dropped on the microchamber, then a glass cover slip was placed over the microchamber array, and the extra water was removed. The water evaporated from the edge of the cover glass by capillary effect and the speed of the disappearance of water was compared. The ability to retain the solution in a microchamber is found to be strongly dependent on the hydrophilic property of the inner wall and the rest of the substrate. The microchamber array is similar to those featured in Figure 2.26 (see Figure 2.27).
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Figure 2.27: Top view of scanning electron microscopy images of part of the microchamber array [6].

Table 2.1 below shows the relationship between the surface condition of the array and the retention time of the water.

Table 2.1: Relationship between surface states of the microchamber array and retention time of water [6].

<table>
<thead>
<tr>
<th>hydrophilicity</th>
<th>retention times/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner walls</td>
<td>substrate</td>
</tr>
<tr>
<td>X</td>
<td>O</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>O</td>
<td>X</td>
</tr>
</tbody>
</table>

*(O) indicates hydrophilicity and (X) indicates hydrophobicity.*

In cases where both substrate and inner walls are bare silicon (hydrophobic), the water is leaked out in several tens of seconds. By depositing SiO$_2$, the substrate and inner walls could be made hydrophilic. It is found that when the inner walls are hydrophilic, the microchamber array can retain water for over 3 hours. A microchamber with hydrophilic inner walls on a hydrophobic substrate has excellent water retention abilities.

Similar to the constant temperature control method used by Kopp et al. [11], Chou et al.[25] has designed and fabricate a miniaturized cyclic PCR device, seen in Figure 2.28. 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 reaction (Reactor 3)
to take place.

![Figure 2.28: Design of the cyclic PCR chip (top) [25].](image)

Sample is manipulated using a peristaltic pump and the device could achieve 30
cycles in 40 min with flow rate of 15 µL/min.

A micro-machined thermal reactor is presented by Zou et al. [21] in which the
silicon substrate of the PCR chip remains unheated during PCR. It has a new
thermal isolation design (see Figure 2.29) to eliminate thermal cross talk between
reactor and the substrate, and to reduce the parasitic heat capacitance, thus the
silicon substrate could remain unheated due to this thermal isolation.

![Figure 2.29: Schematic structure of thermal reactor. Heaters are placed at the small joint areas close to the silicon beams [21].](image)
The heat transfer is dominated by conduction in the silicon beams, resulting in faster cooling rate compared with those using convection heat transfer. Unlike conventional systems, instead of having a heater placed uniformly in contact with the entire reaction chamber, heating is achieved by joint heating (shown in Figure 2.30). With symmetrical thermal arrangements, temperature deviations within the reaction chamber are found to be zero.

![Figure 2.30: Schematic designs of (a) uniform-heating and (b) joint-heating [21].](image)

The specifications of the thermal reactor are:

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating power</td>
<td>1.2 ~ 1.8 W</td>
</tr>
<tr>
<td>Heating voltage</td>
<td>8 V</td>
</tr>
<tr>
<td>Ramping rate</td>
<td>14 ~ 100 °C/s</td>
</tr>
<tr>
<td>Cooling rate</td>
<td>10 ~ 70 °C/s</td>
</tr>
<tr>
<td>T-Deviation @ 100 °C</td>
<td>0.3 °C</td>
</tr>
<tr>
<td>Temperature accuracy</td>
<td>+/-0.2 °C</td>
</tr>
</tbody>
</table>
Zhao et al [63] have designed and fabricated a micro-litre PCR chip which consists of a vessel, platinum thin film heater and a temperature sensor as shown in Figure 2.31.

Figure 2.31: (a) Main structure and dimension of PCR microchip (b) The heater and temperature sensor [63].

The maximal heating speed is over 15°C/s and cooling speed of 10°C/s.

Unlike previous concepts, Oda et al. [10, 26] presented an infrared mediated thermocycler. In this system, heat was generated using infrared radiation, and cooling was effected by use of compressed air at room temperature. The instrumentation for the infrared-mediated thermal cycling is shown in Figure 2.32.

Figure 2.32: Instrumentation for infrared-mediated thermocycling [26].
As seen in Figure 2.32, the heat source can be manipulated through lenses and a filter system mounted between the tungsten lamp and the PCR apparatus, not only to focus the radiation but also to eliminate wavelengths that could interfere with the PCR reaction. Positive cooling is effected by use of a non-contact air source that forces air at or across the vessel. In this case, this air source is a compressed air source. This method utilizes both a non-contact heat source and a non-contact cooling source for rapid thermocycling. In this approach, the heating and cooling rates are not affected by the heating/cooling step of the chamber components and the heating/cooling block. It is therefore capable of eliminating lengthy time lags between temperature stages, thus allowing for very sharp temperature transitions. Temperature control as rapidly as 10°C/s (heating) and 20°C/s (cooling) is attained.

As mentioned, thermal mass problem in thermocycling has been eliminated by using IR heat source to specifically heat the PCR solution. Hence, Giordano et al. [18] developed a polyimide microchip to carry out PCR using an infrared-mediated temperature control. The experimental set-up is shown in Figure 2.33.

Figure 2.33: setup for non-contact heating of PCR chamber [18]
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The lamp, a 50W IR source, was focused on the PCR chamber using lens and then filtered through an IR transmitting black glass filter. A gold-coated mirror is placed above the microchip to reflect IR light back onto the PCR chamber to enhance the rate of heating. In addition, Giodarno et al. [18] designed the polyimide chip as shown in Figure 2.34 below and this layer is sandwiched between two additional layers of polyimide.

![Figure 2.34: The polyimide microchip [18].](image)

The PCR chip requires careful selection of a suitable material that combines favourable surface and thermal properties, both of which must be conducive to fast and efficient thermocycling. In this respect, polyimide is suitable as it has a sufficiently high glass transition temperature of 350°C to retain its structural integrity over the temperature range for thermal cycling. Secondly, it is transparent in the 600 to 3000nm range, thus allowing radiation to heat the solution alone and not the substrate. By experiment, over 60% of the electromagnetic radiation is found to be transmitted through the polyimide PCR chip at wavelengths between 500 and 2700 nm. This resulted in a large percentage of near-IR light in the important wavelength range reaching the PCR mixture and thus enabling rapid heating of small volumes of aqueous mixtures. Heating and cooling rates of 20°C/s are achieved. Therefore, Giodarno et al. [18] had developed a polyimide microchip that is capable of attaining rapid, accurate and reproducible thermal cycling of PCR solution.
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Lagally et al. [108] had developed a fully integrated system in glass for the manipulation, amplification and CE separation of submicroliter volumes of DNA. The mask design for this system is presented in Figure 2.35. It features the microfluidic loading and positioning of sample in closed PCR chambers with the use of active valves and hydrophobic vents, rapid PCR amplification using thin film heaters, followed by direct injection and rapid separation on a microfabricated CE channel.

![Figure 2.35: PCR-CE mask design [108]](image)

From Figure 2.35 (a), the sample can be loaded in the fluidic bus reservoir (A), and travels through valve port (B) into the PCR chamber (C). The sample stops at the vent port (D). The PCR chamber is connected directly to the injection cross and separation channel with its cathode (E), waste (F), and anode (G) reservoirs. Figure 2.35 (b) enlarges the side and top views of the valve and vent structures. This multiple reaction system enables the introduction of a single sample into four
different reactors for multiple analysis. The main advantage of this microfluidic system is its ability to manipulate small sample volume without introducing air bubbles into the system. The large temperature changes of PCR will drive bubble expansion and contraction, which in turn will cause sample movements and localized heating. The hydrophobic vents allow positive and sensorless positioning of the sample during the loading phase. These vents position the sample and degas the reaction. The membrane allows the sample to degas through diffusion during the PCR reaction, as well as provides an escape for bubbles that are formed during thermal cycling. Thus, this device eliminates sample handling after the initial loading of the sample bus, which increases assay speed and reproductivity and reduces the possibility of sample contamination from external sources. Experimentally, this device demonstrated successful DNA amplification from 0.28µl sample volume by performing 20 cycles of PCR in 10 minutes.

Maluf et al. [37] mentioned that the high thermal conductivity of silicon would allow fast thermal cycling. Its capability to produce chambers of high degree of accuracy and uniformity also makes it desirable [38]. However, silicon poses a problem of material compatibility as enzyme binding to the 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 [7,21,38,53,63].

Taylor et al. [33] have demonstrated real-time, homogeneous, sequence specific detection of PCR products in silicon microstructures. PCR performance is found to be comparable to results from standard-tube formats. Rapid thermal cycling is optimised in which the temperature ramp rates of 4°C/s are attained and cycle time is reduced.

Glass is another commonly used material in microfabrication. Taylor et al. [13] also optimised the PCR efficiency in glass microstructures with real time detection to analyse the device performance. 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 include 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 a result, the glass microstructure displays efficient PCR.

Lin et al. [28, 29] demonstrates the use of finite element analysis to simulate the thermal characteristics of the PCR chip with the aim to shorten thermal cycling time of the chip. Based on the results of the simulation, a chip is developed (as seen in Figure 2.36).

![Figure 2.36: Schematic drawing of the DNA sample filled micro PCR chip with a heat source [28].](image)

Subsequently, an improved thermal profile with 5s denaturation and annealing holding time and zero holding time in primer extension was achieved.

A simple, low cost, high-resolution plastic microcasting method is demonstrated by Sethu et al [30] to fabricate a PCR micro-device as seen in Figure 2.37.
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The device consists of a reaction chamber, a resistive heating coil, a thermoelectric (TE) cooler and a thermocouple and is capable of achieving heating rate of up to \(2.4^\circ\text{C}/\text{s}\) and a cooling rate of \(2^\circ\text{C}/\text{s}\).

Shinohara et al [35] has developed a reactor array for PCR. The concept of their development is to attain high throughput from their 96 micro reaction well array (Figure 2.38).

Figure 2.37: Photograph of micro PCR device [30].

Figure 2.38: Schematic (left) and cross sectional view (right) of the micro reactor array [35].
Finite element thermal analysis was performed to optimise the PCR chip design, so as to achieve precise temperature control of each well and efficient temperature control of each well array. As a result, heating rate of 10°C/s is attained with this design and temperature difference in a micro well was kept within 2.2°C.

The Material Science & Technology Cooperation (MATEC) of the University of Bremen, Germany [53] had developed a silicon micro-system, which incorporates a small thermal reactor and a cleaning chamber as seen in Figure 2.39.

![Cross section of a silicon microstructure used for fast amplification and cleaning of DNA sequences](image)

Figure 2.39: Cross section of a silicon microstructure used for fast amplification and cleaning of DNA sequences [53].

This thermal design allows heating and cooling rates of up to 40°C/s, which leads to an overall PCR duration of only 10 to 15 minutes. The cleaning chamber is filled with immobilised micro-particles (also known as beads) to isolate PCR products from the remaining solution. An electromagnet holds the paramagnetic beads by magnetic force while the PCR solution is pumped into the cleaning chamber.

The Institute of Microelectronics (IME), collaborated with National University of Singapore, had developed a PCR chip [31] shown in Figure 2.40 below. This chip is capable of parallel independent thermal control of multiple reaction chambers, unheated substrate during thermal cycling, good temperature uniformity inside the reaction chamber, real-time on-chip monitoring of temperature and its
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distribution, and integrated fluidic control components for manipulating small volume of sample.

![Image](image.png)

Figure 2.40: The one-chamber (left) and the multi-chamber micro PCR chip (right) [31].

Specifications achieved are listed in Table 2.3 below:

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heating power</td>
<td>1~3 Watt</td>
</tr>
<tr>
<td>Cooling</td>
<td>10-70 °C/s</td>
</tr>
<tr>
<td>Cross-talk</td>
<td>&lt; 0.4 °C</td>
</tr>
<tr>
<td>Heating voltage</td>
<td>8 V</td>
</tr>
<tr>
<td>T-uniformity</td>
<td>± 0.3 °C</td>
</tr>
<tr>
<td>Chamber volume</td>
<td>0.1~20μL</td>
</tr>
<tr>
<td>Ramping</td>
<td>14-100 °C/s</td>
</tr>
<tr>
<td>T-accuracy</td>
<td>± 0.2 °C</td>
</tr>
</tbody>
</table>

In addition, Deshpande et al. [32] conducts a CAD analysis of PCR well containment and presents a model to represent the behaviour (such as the thermal cycling characteristics) of the PCR well (see Figure 2.41), which could be implemented into system models for the entire device. This model for PCR well containment is intended to be predictive and could be applied to develop optimal
designs for the wells to reduce/prevent contamination from leakages between neighbouring wells.

![Solid model of PCR wells/array](image)

Figure 2.41: Solid model of PCR wells/array [32].

Their methods are validated by conducting diffusivity experiments in the microchannels and there is an agreement between the experimental and simulated results. Subsequently, this formulation is used to predict the extent of containment during PCR. The simulated time for the diffusion of material out of a microwell and along an interconnect are compiled in Figure 2.42 which could prove to be an important asset in the design of micro-devices since it indicates the allowable reaction times that prevent contamination of reagents between neighbouring wells.

![Contamination graph](image)

Figure 2.42: Containment in well during reaction and contamination from neighbouring well [32].
There are also several reports where PCR has been performed on electrophoresis devices [40]. The PCR material has been incorporated into one or more of the reservoirs of the device and the whole device could be thermally cycled and the products analyzed electrophoretically [59, 60, 61].

2.5.2 Advantages of PCR miniaturization

As seen from the various PCR microchips that have been developed, PCR miniaturization can bring about the following advantages:

1. More precise temperature control
   Microsystems are more suited to precise temperature control than conventional systems due to the short paths for heat transport and its high surface area to volume ratio. Heaters and sensors for local temperature measurement can be located in very close proximity or connected to the chamber containing the sample (for example, thin film platinum temperature sensor). This can provide a more accurate reading on the sample temperature. The choice of geometries and materials allows configurations for well-controlled local heat flow during denaturing, annealing and extension process [19, 64].

2. Faster thermal cycle
   Since the heating element and cooling elements possess small volumes and small heat capacities, achieving high cooling and heating rates of about 15-40 °C/s is possible [18, 19]. Ultimately, the minimum reaction time is limited by the speed of the enzyme itself [9]. In addition, since the sample volumes have shrunk significantly to the range of micro-litre or less, sample thermal cycling becomes faster [18, 40].
3. **Cost reduction**
   The cost of reagent is reduced due to miniscule 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 [34,40].

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

5. **Less waste resulted**
   The coupling of traditional sample preparation with microchip electrophoresis is wasteful. For example, 20-50 µL of PCR product is generated with conventional thermal cycling. However, microchip analysis requires as little as 1 µL of sample with only a few pico-litres actually consumed. In the case of PCR chip, only a small volume of sample is needed for thermal cycling [18].

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 due to sample handling and the risk of infection will be reduced and execution of the analysis will be faster through reduced manual manipulation [26].
Chapter 3  PCR THERMOCYCLER AND CHIP DESIGN

3.1 Design Philosophy

As discussed in the previous chapter, a wide range of analytical devices has demonstrated the feasibility of molecular assays in micro-scale environments. These miniaturized devices are fabricated by micromachining technologies and have a high performance of chemical analysis with respect to smaller amount of sample, higher analysis speed as well as reduced consumption of chemicals. However, most of the PCR chips developed so far are not disposable due to the expensive fabrication cost for these devices of at least a few micro-liters of sample volume (e.g. 5-25 ul) [88].

On the hand, the conventional plastic PCR micro-plate can be low cost, but the thermal speed is normally slow due to the thermal diffusion delay in the sample that is usually in a cone shaped tube. The thermal diffusion distance in the sample and the plastic wall is usually a few millimeters and hence, the resulted thermal delay is very long (e.g. more than 20-30 s). Consequently, after reaching each temperature stage during thermal cycling, a long time had to be spent to allow a balanced uniform temperature in the sample. This is the key reason why conventional PCR machines need 2-3 hours to finish a 30-40 cycles reaction, even for a moderate sample volume of 5-25 ul.

Hence, the design of the PCR chip has to be of low thermal mass to achieve accelerated thermal cycling rate. Furthermore, a disposable and low cost multi-reaction chip will be an added bonus to the PCR chip design. This could be achieved partly due to the thermocycler design, which could utilize an external temperature sensor and external heat transfer mechanism, thus resulting in a simpler chip design and ease in chip fabrication. Based on this design philosophy, the ultimate aim of the proposed design of the PCR microchip and thermocycler is to fulfill the function of PCR amplification.
3.2 Design requirements for PCR System

After the review of previous works and theories of PCR, the following design criteria are highlighted and will be taken into considerations during the PCR chip and thermocycler design.

1. Precise temperature control

Conventional PCR instruments, as mentioned before, often uses a large heating or cooling block, thus requiring only one thermocouple in the heating/cooling block to control temperature of PCR samples. In micro PCR systems, without the large heating or cooling block, the temperature of the PCR solution must be individually controlled in every PCR chamber. Exact or nearly exact maintenance of a reaction temperature is often critical in most biochemical/biological processes to guarantee their successful completion. Exceeding a desired temperature can lead to inaccurate results.

2. Thermal mass of the PCR system

Even though all elements in a PCR chip are small, these elements such as the heat source, the PCR reaction mixture and even the microchip itself will contribute to the thermal mass of the PCR system. Heat must be transferred from the heat source through the PCR chip material to the reaction sample, thus limiting the rates for heating and cooling [18].

3. Leakages

As pointed out by Deshpande et al [32], leakages on the microchip could result in contamination from materials from adjacent reaction chambers. This will create inhibition to PCR. The risk of contamination will be greatly reduced in a well-sealed PCR chip.

4. Bubbles formation

Air bubbles in quantity will interfere with the PCR process. Therefore, the design of the reaction chamber is crucial to achieving a bubble-less filling of the chamber.
5. **Uniformity of sample temperature**
   The uniformity of sample temperature is important to ensure reliable and reproducible results. This is a challenge, as by increasing cycle speeds, the maintenance of homogeneous sample temperature is made more difficult [10].

6. **Cycle times**
   Cycle times are largely defined by how quickly the temperature of the sample can be changed and relate to the heat source itself and the rate of heat transfer to the sample.

7. **Amplification of the desired molecules**
   The extreme sensitivity of PCR increases the probability that false priming or DNA contamination will lead to the amplification of the wrong molecule. A method to reduce this problem is to work in an extremely controlled environment to prevent impurities and to purify the reagents and primers prior to use. However, even in a controlled environment, the sample itself can contain components that interfere with PCR or cause false priming.

8. **Transition time between temperatures**
   As explained in section 2.2.3, slow transition between temperatures would result in inaccurate PCR results. Transition time between the different stages should be minimised to ensure achieving the desirable PCR product.

10. **Costs**
    Low cost is desirable in producing disposable PCR chips. All materials employed in the chip fabrication should be easily available and cheap.

11. **Throughput**
    The ability to perform PCR in parallel at the same time would greatly increase the throughput of the PCR chip. This is also a significant contribution to cost savings.
In summary, the desired PCR chip should have the following features:

- Standard volume of samples can be used (e.g. from 0.5 to 5 µl)
- Multiple reaction chambers
- Ease in sample and reagents dispensing. Reservoirs and vents to allow air to escape during sample injection
- Seals or sealants to prevent evaporation and contamination of PCR reagents
- Low cost; disposable if possible
- Ease of fabrication

The desired PCR thermocycler should include the following features:

- Accurate and fast temperature control
- An external temperature sensing device to simplify PCR chip production
- Components for fast heating and cooling rate
- Applicable for PCR chips of standard size
- On-board real-time PCR products detection

### 3.3 Design specifications

In the initial design stage, the determination of the specifications of the PCR chips is based on the following considerations:

- The PCR chamber volume is initially set as 5-10 µl.
- A PCR chip of few reaction chambers will be fabricated first and tested in the proposed design. Little or no compromise of its performance is ensured and is desirable for practical applications.
- The dimensions of the PCR chip is in the order of $1 \times 1 \text{ cm}^2$, so that it is small enough to be made portable and a lower material cost.
- Temperature ramping for each thermal cycling process should be faster than 4°C/s, which is the ramping rate offered by most conventional PCR systems.
- Able to perform at least 30 cycles of PCR amplification.
3.4 **Conceptual design**

As seen from the design requirements, a faster rate of heating and cooling of the sample within the PCR chip allows a faster transition between different thermal cycling processes and a faster cycle time. This is also one of the key considerations in the development of PCR systems. As seen in the literature review, the thermal mass of the heating/cooling blocks and the material of the PCR chip itself is the main restricting factor for rapid thermal cycling. Many developed PCR systems aim to eliminate or reduce the thermal mass. The following proposed concept is designed with this motive in mind in order to achieve the desired heating and cooling rate.

The conceptual design for the PCR chip is presented in Figure 3.1 below. A chip design of similar concept has been developed and patented by another research group in Nanyang Technological University (NTU, Singapore). It has an array of reaction chambers, connected by two main channels. The total area occupied by the chip is equivalent to the size of a standard microscopic glass slide, as different chip design can be easily fabricated on the glass slide by MEMS techniques.

![Conceptual design of PCR chip](image-url)

Figure 3.1: Conceptual design of PCR chip (not to scale)
Chapter 3: PCR THERMOCYCLER AND CHIP DESIGN

Channels and chamber depths are in the order of micrometers. PCR mix could be injected into the dispensing reservoir, which in turn fills the individual PCR reaction chambers via Channel 1. Capillary action enables the PCR mix to flow into the reaction chambers, and prevents the solution to flow into Channel 2.

Capillary action is a physical effect caused by the interactions of a liquid with the walls of a thin tube. In this case, PCR mix enters the smaller chambers because of the strong hydrogen-bonding interactions between the water molecules in the mixture and the oxygens (and terminal hydrogens) at the surface of the glass ($\text{SiO}_2$; surface oxygens are typically bonded to hydrogen).

It is this strong bonding interaction, which creates an upward force on the liquid at the edges and result in a meniscus that turns upward. The surface tension acts to hold the surface intact, so instead of just the edges moving upward, the whole liquid surface is dragged upward.

The narrower the tube, the higher the liquid will climb, because a narrow column of liquid weighs less than a thick one. Similarly, this strong hydrogen-bonding interactions prevents the PCR mix to flow into Channel 2. In addition, by ensuring that the chambers are made of hydrophilic materials, frictional forces along the chamber walls will be smaller than the surface tension of the fluid, thus the fluid can flow into the reaction chambers more easily.

Lastly, both Channel 1 and 2 are filled with sealant to prevent sample contamination and evaporation during PCR. This sealant could be molecular grade mineral oil [19] or silicone glue that is suited for molecular work. Excess PCR mix remaining in Channel 1 will be flushed out by this sealant and collected at the waste reservoir. Lastly, PCR chip undergoes PCR.

3.5 Investigation of the methods of heat transfer
The discipline of heat transfer is concerned with only two things: temperature, and the flow of heat. Temperature represents the amount of thermal energy available, whereas 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. The greater a material's temperature, the greater the thermal agitation of its constituent molecules (manifested both in linear motion and vibration modes). 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 differing temperatures. Examples include thermal conductivities, specific heats, material densities, fluid velocities, fluid viscosities and surface emissivities. There are 3 categories of heat transfer mechanisms, namely conduction, convection and radiation.

3.5.1 Conduction heat transfer

When there exists a temperature gradient within 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,

\[ q = -k \nabla T \]  

(3.1)

This equation determines the 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.

The temperature profile within a body depends upon the rate of its internal-generated heat, its capacity to store some of this heat, and its rate of thermal conduction to its boundaries (where the heat is transferred to the surrounding environment).

\[
\text{Net rate of heat gain by conduction} + \text{Rate of energy generation} = \text{Rate of increase of internal}
\]

Mathematically, this is stated by the heat conduction equation,
Chapter 3: PCR THERMOCYCLER AND CHIP DESIGN

\[
\nabla^2 T + \frac{1}{k} q_{gen} = \frac{1}{\alpha} \frac{\partial T}{\partial t}
\]

(3.2)

In the heat conduction equation, the power generated per unit volume is expressed by \( q_{gen} \). The thermal diffusivity \( \alpha \) is related to the thermal conductivity \( k \), the specific heat \( c \), and the density \( \rho \) by,

\[
\alpha = \frac{k}{\rho c}
\]

(3.3)

3.5.1.1 Conduction heat transfer in PCR microchips

Heating/cooling block is commonly used in PCR devices to rapidly conduct heat to/away from the PCR sample. This heating/cooling block is usually in intimate contact with PCR reaction chamber [7, 17]. As mentioned before, the thermal mass of the heating/cooling block restricts the rate of heat transfer between the sample and the block. Heat conduction is also the basis of most commercially PCR instrumentation.

For this form of heat transfer, surface to volume ratio of the reaction vessel is of utmost importance to the effectiveness of thermal cycling; the higher this ratio, the better the PCR reaction. There are instances of this problem being reduced/eliminated. For example, Nagai et al. [17] has demonstrated that having the PCR sample to move to three different regions of constant temperatures could eliminate the problem of large thermal mass of the heating/cooling block.

In addition, Zou et al. uses silicon beams to form a thermal path between the reaction chamber and the substrate. Heat is conducted directly between the sample and heater through the silicon beams [21], thus the reaction chamber is not heated. This reduces the thermal mass required to heat up the sample and also improves response time.
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Other limitations of using conduction heat transfer is highlighted in [10] in which the initial design of the PCR chip is largely predetermined by the heating/cooling element as the location of the element is typically a part of the chip. Thus, these micro-devices could become spatially constrained and the devices are not flexible with respect to the use of heating or cooling on different locations within or at the micro-device structure. In addition, the design of single-use analyses modules for various diagnostic and monitoring purposes with integrated heating/cooling element could be very complex and becomes cumbersome. When numerous samples are being tested, it could also be difficult to use. Therefore, these factors compromise the advantage of using inexpensive micro-fabrication technology to produce such devices.

3.5.2 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 temperature of the solid due to an external field such as fluid buoyancy can induce a fluid motion. This is known as natural convection and it is a strong function of the temperature difference between the solid and the fluid. Blowing air over the solid by using external devices such as fans and pumps can also generate a fluid motion. This is known as forced convection.

The essential ingredients of forced convection heat transfer analysis are given by Newton's Law of Cooling,

\[ Q = hA(T_w - T_\infty) = hA \cdot \Delta T \]  

(3.4)

The rate of heat transferred to the surrounding fluid is proportional to the object's exposed area \( A \) and the difference between the object temperature \( T_w \) and the fluid free-stream temperature \( T_\infty \). The constant of proportionality \( h \) is termed as the convection heat transfer coefficient. Other terms describing \( h \) include film
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coefficient and film conductance. Similar to forced convection, heat transfer due to free convection is also described by Newton's Law of Cooling (50).

The viscosity of the fluid requires that the fluid have zero velocity at the plate's surface, thus a boundary layer exists where the fluid velocity changes from \( \mu_{\infty} \) 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 \( \text{Re} \) of the flow is sufficiently high.

The transition from laminar to turbulent for flow over a flat plate occurs in the range,

\[
3 \times 10^5 < \text{Re}_x < 3 \times 10^6, \quad \text{Re}_x = \frac{\rho u_{\infty} x}{\mu} \tag{3.5}
\]

Thus, laminar flow will have \( \text{Re}_x < 3 \times 10^5 \) while turbulent flow occurs when \( \text{Re}_x > 3 \times 10^6 \).

### 3.5.2.1 Convection heat transfer in PCR microchips

Thermal cycling by convection is usually done by rapidly heating or cooling a circulating medium such as air to affect the temperature of the PCR reaction chamber. In this manner, the reaction sample within the chamber will be subjected to the desired thermal cycling process. Hence, temperature cycling is conducted with small volume samples without physical contact between the heat source and the sample.

For instance, Oda et al. [26] uses a non-contact air source that forces air at room temperature across the reaction vessel to specifically effect cooling.

Similar to conduction heat transfer, heat transfer between the sample and heat source depends on direct contact between the heat source (e.g. air or water) and the reaction chamber walls. Therefore, the surface to volume ratio of the reaction vessel determines the effectiveness of thermal cycling,
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such that the higher this ratio is, the better the PCR reaction. A limitation of the convective heat transfer is that if the device is being heated/cooled in an uncontrolled manner through heat absorption/dissipation at ambient temperature, the ambient temperature restricts the maximum/minimum temperature at which the device can operate. This restriction is particularly inconvenient and restricts the usefulness of convective heat transfer [10].

3.5.3 Radiation heat transfer

Radiation heat transfer is concerned with the exchange of thermal radiation energy between two or more bodies. Thermal radiation is defined as electromagnetic radiation in the wavelength range of 0.1 to 100 microns and arises from a temperature difference between 2 bodies. No medium needs to exist between the two bodies for heat transfer to take place (as is needed by conduction and convection). Rather, the intermediaries are photons, which travel at the speed of light.

Radiation heat transfer must account for both incoming and outgoing thermal radiation. Incoming radiation can be absorbed, reflected, or transmitted. This decomposition can be expressed by the relative fractions,

\[ 1 = \rho + \alpha + \tau \]  \hspace{1cm} (3.6)

where \( \rho \) is the surface’s reflectivity, \( \alpha \) is the material’s absorptivity and \( \tau \) is its transmissivity.

To account for a body’s outgoing radiation (or its emissive power, defined as the heat flux per unit time), a comparison was made with a perfect body that emits as much thermal radiation as possible. This perfect body is known as a blackbody, and the ratio of the actual emissive power \( E \) to the emissive power of a blackbody is defined as the surface emissivity \( \varepsilon \),

\[ \varepsilon = \frac{E}{E_{\text{blackbody}}} \]  \hspace{1cm} (3.7)
Subsequently, the heat emitted by a blackbody (per unit time) at an absolute temperature of \( T \) is given by the Stefan-Boltzmann Law of thermal radiation,

\[
\dot{Q} = A\sigma T^4 = AE_{\text{blackbody}}
\]

where \( Q \) has units of Watts, \( A \) is the total radiating area of the blackbody, and \( \sigma \) is the Stefan-Boltzmann constant.

Bodies that emit less thermal radiation than a blackbody have surface emissivities \( e \) less than 1. If the surface emissivity is independent of wavelength, then the body is called a "gray" body, in that no particular wavelength (or color) is favored. The net heat transfer from a small gray body at absolute temperature \( T \) with surface emissivity \( e \) to a much larger enclosing gray (or black) body at absolute temperature \( T_e \) is given by,

\[
Q = e A\sigma (T^4 - T_e^4)
\]

For the case where there are more than two surfaces, view factors \( F \) must be introduced. View factor is defined as the fraction of the radiation leaving surface \( i \) that is intercepted by surface \( j \). From the definition of the view factor, the summation rule

\[
\sum_{j=1}^{N} F_{ij} = 1
\]

may be applied to each of the \( N \) surfaces in the enclosure. This rule follows from the conservation requirement that all radiation leaving surface \( i \) must be intercepted by the enclosure surfaces.

The case of two bodies in thermal equilibrium can be used to derive the following reciprocity relationship for view factors.

\[
A_i F_{ij} = A_j F_{ji}
\]
This reciprocity relationship is useful in determining one view factor from the knowledge of the other. Radiation view factors can be analytically derived for simple geometries and are tabulated in several references on heat transfer [48, 49, 50]. They range from zero (e.g. two small bodies spaced very far apart) to 1 (e.g. one body is enclosed by the other).

3.5.3.1 Net radiation heat transfer at a surface in an enclosure

A major complication associated with radiation exchange between non-black surfaces is due to surface reflection. In an enclosure, radiation may experience multiple reflections off all surfaces with partial absorption occurring at each surface. Analysing radiation exchange in an enclosure may be simplified by making certain assumptions. Each surface of the enclosure is assumed to be isothermal and to be characterised by a uniform radiosity and irradiation. The medium within the enclosure is also taken to be non-participating.

The net effect of radiative heat transfer occurring at the surface, \( Q_i \), is given as

\[
Q_i = A_i (J_i - G_i)
\]  

(3.12)

The definition of radiosity, \( J_i \) is,

\[
J_i = E_i + (\rho_i + \tau_i)G_i
\]  

(3.13)

where \( E \) is the surface emissive power, \( \tau \) is the transmissivity of the surface, \( \rho \) is the reflectivity of the surface and \( G \) is the irradiation on the surface. Radiosity is a term that accounts for all the radiant energy leaving a surface, which includes the reflected portion of the irradiation, as well as direct emission. If the emissive power exceeds its radiosity, there is net radiation heat transfer from the surface; if the inverse is true, the net transfer is to the surface [49].

Equation (3.13) may also be expressed as

\[
J_i = \varepsilon_i E_{im} + (\rho_i + \tau_i)G_i
\]  

(3.14)
Solving for $G_i$,

$$ G_i = \frac{J_i - \varepsilon_i E_{bl}}{\rho_i + \tau_i} \quad (3.15) $$

Substituting (3.15) into equation (3.12), we have

$$ \dot{Q}_i = A_i \left[ \frac{(\rho_i + \tau_i - 1) J_i + \varepsilon_i E_{bl}}{\rho_i + \tau_i} \right] \quad (3.16) $$

The net rate of radiation from surface $i$, $q_{in}$, to the sum of components $q_{ij}$, related to radiative exchange with the other surfaces, as seen in Figure 3.2 below.

![Figure 3.2: Network representation of radiative exchange between surface i and remaining surfaces of an enclosure [49]](image)

Hence,

$$ \dot{Q}_i = \sum_{j=1}^{N} A_i F_{ij} (J_i - J_j) = \sum_{j=1}^{N} q_{ij} \quad (3.17) $$
By combining equations (3.16) and (3.17), we have

\[ A_i \left[ (\rho_i + \tau_i - 1)\gamma_i + \varepsilon_i E_{bl} \right] = \sum_{j=1}^{N} \frac{J_i - J_j}{(A_i F_j)^{-1}} \]  

(3.18)

3.5.3.2 Radiation heat transfer in PCR microchips

To achieve radiation heat transfer in PCR chip applications, radiant energy is generated using an IR source that is not in direct contact with the sample vessel. This energy could be easily and accurately manipulated, so that its intensity, which is directed towards the sample, can be finely tuned and controlled. Therefore, the desired temperature could be achieved and maintained. Unlike convection and conduction heat transfer, heating by radiation mainly affects the sample itself, rather than the reaction vessel and/or its environment, thus more rapid transition from one to another temperature in IR-mediated heating is possible. The IR-mediated heating is primarily achieved through the absorbance of irradiation by molecules of the sample [10]. Although some heating of the sample might be the result of the reaction vessel itself absorbing the irradiation of the IR light, heating of the sample is still primarily caused by the direct action of the IR wavelengths on the sample itself. The material for borosilicate glass and its IR transmissivity is 0.92 @ 2 um wavelength [92]. Hence, up to 92% of the radiated heat from the heat source, that is incident on the capillary, would be directed to the sample itself.

Oda et al. [26] has illustrated the use of a tungsten lamp as an ideal and inexpensive non-contact heat source. It could almost instantaneously reach very high temperatures (internal lamp temperature of ~3500K reached in milliseconds). The concept of using an IR radiation source that would specifically heat the sample without having to heat the medium surrounding the vessel or the vessel itself distinguishes this approach from previously described approaches. Heat transfer problems associated with
solid block heaters are basically eliminated with this non-contact approach.

3.5.4 Alternative heating methods

The rationale of potential techniques such as microwave heating and laser heating would be addressed here. An experiment conducted by Kakita et al. (1995) indicates that microwave could destroy the DNA by causing DNA covalent bonds to break. This would not have occurred from heating alone [70]. However, it was not proven whether microwave has directly caused the covalent bond breakage. It is possible that microwaves may be causing the generation of cupric ions and hydroxyl radicals and the auto-oxidation of amino sugars, which are involved in the DNA strand breakage [71].

On the other hand, laser radiation may lead to formation of cross-links in the DNA, which induce genotoxic effects that are harmful to DNA. In addition, the photothermic processes caused by laser radiation could lead to reduction in enzymatic activity (at 50 °C). Since PCR is a reaction based on the activity of the polymerase enzyme, this photothermic process will affect the yield of PCR [72].

3.5.5 Selection of the heat transfer mechanism

Radiation heat transfer is chosen for the heating stages of PCR as it offers the possibility of achieving the fastest rate of heating while having precise temperature control. This is because heating by IR radiation could specifically heat the sample only, with minimum radiation absorbed by the reaction vessel. This allows more efficient heating as compared to both conduction and convection, which requires heat to be transferred across the reaction vessel. Similar to light, IR waves from light bulb can be manipulated to focus on structures of small shapes and sizes with the aid of optical lenses and filters. Furthermore, without considerations for the inclusion of the heating element within the PCR chip, chip design could be simplified. This would also allow
simpler fabrication processes as well as less materials and fabrication costs. The PCR chip could be made disposable as well.

For cooling during the annealing process, forced convection heat transfer resulted from the use of a non-contact air source that forces air at or across the vessel is selected to achieve cooling. Air at room temperature would be blown across the PCR microchip with the use of fans to enhance heat loss from the substrate. Preferably, the reaction vessel or container will have a high surface-to-volume ratio. A high surface-to-volume ratio leads to an increase in the efficiency of the thermocycling and also can lead to the effectiveness of the cooling step. Consequently, heat loss is achieved primarily by forced convection.

Because of the non-contact heating and cooling sources, the reaction vessel is the only part of the apparatus that needs to be changed with each sample. Thus, rapid and accurate thermocycling of numerous samples can be performed in an economic manner.

3.6 Material selection
PCR chips can be constructed out of a multitude of materials depending on their unique characteristics and properties. Since radiation heat transfer is selected to provide rapid heating, the sample vessel must be made of a material that allows the penetration of IR light wavelengths. Examples of such materials are quartz glass, silicon and transparent plastics [26]. Thus, the following materials discussed are either transparent or partially transparent to infrared radiation, and are possible choices for fabricating a PCR chip.

3.6.1 Silicon
Exploitation of silicon as a substrate for micro-machined devices is well established in the engineering fields. It is the most common material used in semiconductor devices. In biomedical applications, silicon has been used as a substrate to develop devices for pH measurement and biosensors. Other
applications have exploited the thermal conductivity of silicon to construct thermal cycling devices for use in PCR [7].

Silicon is partially transparent to infrared radiation [42]. It is a good thermal conductor and its thermal conductivity has been exploited to construct thermal cycling devices for use in PCR [7]. Due to its atomic similarities to diamond, single crystal silicon is a very hard substance. It exceeds the mechanical strength of steel, but is decidedly more brittle. Its strength makes silicon ideal for many MEMS structures, as it has the highest fracture strength of any material commonly used in MEMS.

Well-controlled processes will yield high purity crystalline silicon structures that are desirable as its mechanical properties can be very reproducible. For these reasons, silicon is often used for high-quality microstructures. A minor drawback of silicon is that it does not have the superior electrical and optical properties of other materials. Properties of silicon are listed below in Table 3.1.

<table>
<thead>
<tr>
<th>Properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>2320 kg/cm²</td>
</tr>
<tr>
<td>Young's modulus</td>
<td>107 GPa</td>
</tr>
<tr>
<td>Coefficient of thermal expansion</td>
<td>$4.2 \times 10^{-6} \text{°C}^{-1}$</td>
</tr>
<tr>
<td>Heat capacity</td>
<td>0.705 J/g·°C</td>
</tr>
<tr>
<td>Thermal conductivity</td>
<td>150 W/m·K</td>
</tr>
<tr>
<td>Melting point</td>
<td>1412 °C</td>
</tr>
<tr>
<td>Refractive index</td>
<td>3.42</td>
</tr>
</tbody>
</table>

Table 3.2: Optical properties of silicon (1 mm thick) @ 4 um [15,42,69].

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorptivity</td>
<td>0.092</td>
</tr>
<tr>
<td>Transmissivity</td>
<td>0.558</td>
</tr>
<tr>
<td>Reflectivity</td>
<td>0.350</td>
</tr>
</tbody>
</table>
3.6.2 Glass

Glass is a simple fusion of sand, soda & lime (all opaque), which produces a transparent "solid" when cooled. Glass has recently found new applications in the fields of high technology, particularly semiconductor and microelectronics industry, where silica is widely used as an insulator in transistors and in the fiber optics cable industry where high purity silica glass has made advanced telecommunications possible.

A frequently used glass is borosilicate glass, which is commonly used to make scientific glassware such as beakers, test tubes, capillaries and microscopic slides.

Borosilicate glass is far stronger than the glass normally used in our everyday life. In addition, glass does not show a distinct melting point. This means that glass gradually softens on heating and gradually thickens on cooling. It has high a high melting temperature and is a good electrical and thermal insulator.

Table 3.3: Properties of borosilicate glass [49, 68].

<table>
<thead>
<tr>
<th>Properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>2230 kg/cm³</td>
</tr>
<tr>
<td>Young's modulus</td>
<td>64 GPa</td>
</tr>
<tr>
<td>Coefficient of thermal expansion</td>
<td>$3.3 \times 10^{-5} ^\circ C ^{-1}$</td>
</tr>
<tr>
<td>Heat capacity</td>
<td>0.835 J/g.ºC</td>
</tr>
<tr>
<td>Thermal conductivity</td>
<td>1.2 W/m-K</td>
</tr>
<tr>
<td>Melting point</td>
<td>800 ºC</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.473</td>
</tr>
<tr>
<td>Emissivity</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Table 3.4: Optical properties of borosilicate glass (0.13 mm thick) @ 2 um [67, 68, 92]

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorptivity</td>
<td>0.002</td>
</tr>
<tr>
<td>Transmissivity</td>
<td>0.92</td>
</tr>
<tr>
<td>Reflectivity</td>
<td>0.078</td>
</tr>
</tbody>
</table>
3.6.3 Polyimide

Polyimides are a class of organic films that have proven promising as a possible replacement for SiO$_2$ as an insulator in microelectronics. Their main application in MEMS has been in circuits and as a layer of chemically active sensor materials on membranes and cantilevers. Polyimides are weak materials and are generally not considered for structural applications. As good insulators, polyimide films have a myriad of possible uses in the semiconductor industry. Giordano et al. [18] have successfully demonstrated the use of infrared radiation heat transfer on a polyimide PCR microchip and heating and cooling rates of 20°C/s are achieved.

Polyimides are normally infusible, coloured (often amber) high performance polymers with predominantly aromatic molecules of high thermal stability. They have excellent high temperature properties and radiation resistance, inherently low flammability and smoke emission, low creep and high wear resistance and are very expensive. They have moderately high water absorption and are prone to hydrolysis and attack by alcalis and concentrated acids. The material properties of a polyimide film are offered below (Table 3.5).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unit</th>
<th>Muscovite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>g/cm$^3$</td>
<td>1.42</td>
</tr>
<tr>
<td>Young’s modulus</td>
<td>GPa</td>
<td>7.5 - 15</td>
</tr>
<tr>
<td>Heat capacity</td>
<td>J/g-°C</td>
<td>1.09</td>
</tr>
<tr>
<td>Coefficient of expansion per °C perpendiculat to cleavage plane</td>
<td>K$^{-1}$</td>
<td>30 x 10$^{-6}$ - 60 x 10$^{-6}$</td>
</tr>
<tr>
<td>Maximum operating temperature</td>
<td>°C</td>
<td>250 - 320</td>
</tr>
<tr>
<td>Thermal conductivity</td>
<td>W/m-K</td>
<td>0.1 - 0.35</td>
</tr>
<tr>
<td>Emissivity</td>
<td></td>
<td>~ 0.9 [46]</td>
</tr>
</tbody>
</table>

3.6.4 Mica

Mica consists of silicon (Si) and oxygen (O) together with other elements, most commonly aluminium, sodium, calcium, iron, or magnesium. It is a group of
complex aluminosilicate minerals having a sheet or plate like structure with
different composition and physical properties.

Mica is transparent, optically flat, splittable into thin films along its cleavage,
colourless in thin sheets, resilient and incompressible. It is stable and completely
inert to the action of water, acids (except hydro-fluoric and concentrated
sulphuric) alkalies conventional solvents and oil and are virtually unaffected by
atmospheric action. However, it is relatively soft and can be hand cut, machined
or die-punched. It is flexible, elastic and tough, having high tensile strength.

This material is fireproof, infusible, incombustible and non-flammable and can
resist temperatures of 600°C to 900°C, depending on the type of mica. It has low
heat conductivity, excellent thermal stability and may be exposed to high
temperatures without noticeable effect.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unit</th>
<th>Muscovite (a type of mica)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td></td>
<td>Ruby / Green</td>
</tr>
<tr>
<td>Density</td>
<td>g/cm³</td>
<td>2.6 – 3.2</td>
</tr>
<tr>
<td>Heat capacity</td>
<td>J/g°C</td>
<td>0.88</td>
</tr>
<tr>
<td>Coefficient of expansion per °C</td>
<td></td>
<td>9 x 10⁻⁴ - 36 x 10⁻⁴</td>
</tr>
<tr>
<td>perpendicular to cleavage plane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum operating temperature</td>
<td>°C</td>
<td>500 – 600</td>
</tr>
<tr>
<td>Thermal conductivity</td>
<td>W/m-K</td>
<td>0.16 x 10⁻⁴</td>
</tr>
<tr>
<td>Emissivity</td>
<td></td>
<td>0.75</td>
</tr>
<tr>
<td>Moisture absorption</td>
<td></td>
<td>Very low</td>
</tr>
<tr>
<td>Acid reaction</td>
<td></td>
<td>Affected by hydrofluoric acid</td>
</tr>
</tbody>
</table>

Table 3.6: Properties of mica [39]

| Absorptivity | 0.09 |
| Transmissivity | 0.88 |
| Reflectivity | 0.03 |

Table 3.7: Optical properties of mica (0.08mm thick) @ 4 um [69]
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3.6.5 Choice of material

Glass is selected as the material of choice for the proposed design for PCR reaction chamber. This is mainly due to its high infrared transmissivity as compared to silicon and mica. Therefore, more radiated heat could be transmitted through the glass walls to the PCR mixture with little radiation absorbed by the material (only about 0.2% of total radiation is absorbed by glass). Hence, effective IR-mediated heating can occur when the vessel containing the solution will not significantly absorb the transmitted light and allows direct heating of the solution. IR heating of the solution is performed by the excitation of the vibrational bands of water through the IR radiation emitted from the lamp [26]. Glass, being transparent, would also allow for easy monitoring of the PCR reaction occurring within the PCR chip.

Another advantage is that glass has high stiffness (Young's modulus of 94 GPa), which is important to a structure of micrometer thickness. A structure of micrometer thickness, with insufficient stiffness could easily fracture when subjected to a little pressure or force, such as surface tension of moving fluids within the structure. This will ensure a well-sealed reaction chamber environment and is especially important in the design of the PCR chip.

As mentioned before, the proposed PCR thermocycler design is capable of performing PCR on any PCR chip design that is identical in size to the standard microscopic glass slide. Hence, the different materials investigated in this section are feasible options for the materials in PCR chip design due to their IR transmitting properties.

3.7 Examination of temperature sensing methods

Since PCR is a temperature dependent reaction, an accurate method for temperature sensing is important for precise temperature detection and control. Temperature sensor types can be simply classified into two groups, contact and non-contact. These two types of sensors require some assumptions and inferences
to measure temperature. Many well-known uses of these sensors are very 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 the apparent temperature quite different from the indicated temperature. The following sections will discuss on the different types of temperature sensors that could be used in the application of a PCR chip.

### 3.7.1 Contact sensors

Contact temperature sensors measure their own temperature. One infers the temperature of the object to which the sensor is in contact by assuming or knowing that the two are in thermal equilibrium, that is, there is no heat flow between them. Contact temperature sensors come in a variety of types and constructions and are presented here.

**Thermocouples**

Thermocouples are among the easiest temperature sensors to use. 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 the open pair according to the size of the temperature difference between the ends, the relative Seebeck coefficient of the wire pair and the uniformity of the wire-pair relative Seebeck coefficient. The thermocouple will generate electricity when heat is applied to the tip of it.

**Resistance Temperature Detectors (RTDs)**

RTDs are based on the positive temperature coefficient of electrical resistance. RTD elements are wire windings or other thin film serpentinales 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.

**Thermistors**
Chapter 3: PCR THERMOCYCLER AND CHIP DESIGN

Thermistors are embedded 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.

Thermochromic Liquid Crystals
Thermochromic Liquid Crystals (TLCs) are materials that change their molecular structure and optical properties with temperature [55]. This type of liquid crystal used in the temperature-sensing devices is known as twisted nematic liquid crystals. In twisted nematic liquid crystals, the layers contain the long axes of the molecules. Furthermore, the long axes rotate by a small angle from one layer to the next (see Figure 3.3).

Figure 3.3: Arrangement of molecules in a twisted nematic liquid crystal [57].

At temperatures below the TLC's event temperature (temperature at which the TLC begins to reflect visible light), a TLC will be in the solid state and will appear transparent. As the temperature of the liquid crystal rises through the TLC's bandwidth (temperature range in which the TLC actively reflects visible light), the spacing between layers also changes. The change in spacing changes the wavelength of the reflected light and its observed colour. Therefore, the colour of the reflected light is an indication of the temperature of the liquid crystal. Finally, when the temperature exceeds the TLC's clearing point temperature (temperature at which the TLC ceases to reflect visible light), the material will enter the pure liquid state and will revert back to being transparent.
3.7.2 Non-contact sensors

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 the 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. This group of sensors can be divided into several broad categories, such as, both spot and point measuring devices, line measuring radiation thermometers and thermal imaging thermometers.

Spot measuring thermometers

Spot measuring thermometers are intended to measure the temperature of a spot at some distance. These are the most common types of radiation thermometers. They can be further subdivided into various types, depending on whether they are portable (hand-held) or fixed mounted and according to their technical capabilities. These thermometers collect and measure thermal radiation in a single spectral region, thus are also known as single waveband devices and sometimes also known as spot radiometers. Devices measuring in two wavebands simultaneously are called Two Colour Thermometers (pyrometers) or Ratio Thermometers.

Line measuring thermometers

These sensors measure a linear region over a defined angular range. If there's an object of sufficient temperature covered by that angle, then the device produces a linear trace along the line "seen" by the sensor. Some line measuring thermometers are connected to computers in such a way, that if the object moves perpendicular to the measured line, a series of temperature profiles can be connected together to form a sample of the two-dimensional temperature distribution on the object.
Area measuring thermometers

This type of thermometers is commonly known as thermal imagers. They comprise of a detector and lens, and are capable of giving a visual representation of infrared energy emitted by all objects above 0 degrees Kelvin. In other words thermal imagers enable the user to "see" heat.

Thermal imagers could translate the thermal energy, which is transmitted in the infrared wavelength (1 micron to 100 microns) into data that can be processed into a visible light spectrum video display. Depending on the sophistication of your system, thermography is capable of providing very detailed images of situation that is otherwise invisible to the naked eye.

3.7.3 Comparison between the main types of temperature sensors

<table>
<thead>
<tr>
<th>Temperature Sensor Attributes</th>
<th>Thermocouple</th>
<th>RTD</th>
<th>Thermistor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Criteria</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Cost-OEM Quality</td>
<td>Very wide</td>
<td>Short to medium</td>
<td></td>
</tr>
<tr>
<td>Temperature Range</td>
<td>-450°F to +4200°F</td>
<td>-400°F to +1200°F</td>
<td>-100°F to +500°F</td>
</tr>
<tr>
<td>Interchangeability</td>
<td>Good</td>
<td>Excellent</td>
<td>Poor to fair</td>
</tr>
<tr>
<td>Long-term Stability</td>
<td>Poor to fair</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Medium</td>
<td>High</td>
<td>Medium</td>
</tr>
<tr>
<td>Repeatability</td>
<td>Poor to fair</td>
<td>Excellent</td>
<td>Fair to good</td>
</tr>
<tr>
<td>Sensitivity (output)</td>
<td>Low</td>
<td>Medium</td>
<td>Very high</td>
</tr>
<tr>
<td>Response</td>
<td>Medium to fast</td>
<td>Medium</td>
<td>Medium to fast</td>
</tr>
<tr>
<td>Linearity</td>
<td>Fair</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Self Heating</td>
<td>No</td>
<td>Very low to low</td>
<td>High</td>
</tr>
<tr>
<td>Point (end) Sensitive</td>
<td>Excellent</td>
<td>Fair</td>
<td>Good</td>
</tr>
<tr>
<td>Lead Effect</td>
<td>High</td>
<td>Medium</td>
<td>Low</td>
</tr>
<tr>
<td>Size/Packaging</td>
<td>Small to large</td>
<td>Medium to small</td>
<td>Small to medium</td>
</tr>
</tbody>
</table>

Table 3.8: Temperature sensor comparison guide [79]
3.7.4 **Selection of an appropriate temperature-sensing device**

Even though infrared heating is the preferred heating mechanism, non-contact infrared thermometer is not suitable as the background radiation could seriously affect the accuracy of temperature readings, especially when in a small enclosure. A possible remedy to this problem is to raise the PCR microchip surface emittance by applying very small dots of IR-black ink [42]. In addition, the temperature measurement of the PCR reaction chamber would require high measuring resolution due to the size of the reaction chamber. This could be achieved from additional expensive and complex equipments.

On the other hand, the thermocouple would make a good temperature sensor for the PCR chip. From comparison of Table 3.8 and 3.9, the thermocouple is a simple and low cost temperature sensor. It also provides a very wide temperature

<table>
<thead>
<tr>
<th>Table 3.9: Advantages and disadvantages of temperature sensors [79]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensor</strong></td>
</tr>
</tbody>
</table>
| 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  
- Small $\Delta$  
- 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 |
range, moderately fast response and excellent point sensitivity. It is readily available commercially. In addition, thermocouple has no self-heating, as compared to other sensors which could result in an inaccurate detection. Furthermore, thermocouple is an external contact temperature sensor, hence there is no need for embedding costly RTDs or thermistors within the chip and simplifies the chip fabrication process.

3.8 Conceptual design development

3.8.1 PCR chip design

Based from earlier selections, the PCR chip concept is further developed, as seen in Figure 3.4 and 3.5. The following proposed PCR chip design is a capillary array. The dimensions for both the PCR chip and system are based on the volume of PCR mix used (~7µl). A unique feature of this chip design is that it utilizes an array of capillaries, as opposed to micro-machined microchannels and reaction chambers that are both time-consuming and costly. Capillaries are an inexpensive alternative for the containment of the PCR mix, as they are readily available in bulk commercially. They have lengths (ranging from 20mm to 100mm), diameters (from I.D. of 0.05 to 2mm) and standard wall thickness, ranging from 0.015mm to 0.1mm [91]. These capillaries are high precision glass products and are often used for molecular work.

In Chapter 2, Kalinina et al. [22] have demonstrated that PCR can be performed on capillaries successfully. In addition, Taylor et al. [13] have concluded that glass microstructure displays efficient PCR. Hence, capillaries are a feasible reaction vessel for PCR runs.

During convection heat transfer in annealing stage, heat transfer would be improved due to the thin capillary wall. As for heating by radiation, the borosilicate glass capillaries have a high infrared transmissivity (see Table 3.4) of up to 92%. This would allow most of the radiated heat to be transmitted through the capillary wall material with minimal radiated heat absorption and reflection,
thus allowing the PCR mixture to receive most of the heat. In addition, capillaries have a high surface area to volume ratio, which contributes greatly to fast temperature ramping rate and good temperature uniformity. These vessels are typically very inexpensive to purchase and prepare.

Figure 3.4: (a) Plan view of proposed PCR chip design, (b) Cross-sectional view of proposed PCR chip design alongline y-y and (c) Cross-sectional view of proposed PCR chip design alongline x-x. (not to scale)
As mentioned in section 3.4, the size of the PCR chip is equivalent to a standard microscopic glass slide, which is 75mm x 25mm. Light blue regions define the channel walls as well as for holding the capillaries in place. These could be made of quick-dry and easy-to-use adhesive sealant, which can be peeled off easily for disposal after each PCR experiment. Possible sealants/adhesives are high temperature resistance silicone glue and Polydimethylsiloxane (PDMS) elastomer [98, 102, 103]. In addition, the appropriate capillary-to-capillary distance, internal capillary diameters and capillary wall thickness are important chip design considerations and will be examined in the subsequent Chapters 4 and 5.

### 3.8.2 PCR thermocycler design

The thermocycler is designed with a standard glass slide sized PCR chip in mind. This is because different designs of PCR chip can be easily fabricated on a standard glass slide, such as using MEMS technologies. This can be seen from the different PCR chips developed by other researchers, for instance, Zou et al. [21] and Zhao et al [63]. Hence, greater flexibility can be achieved on this thermocycler as it can serve as a test platform for new PCR chip designs. The thermocycler design will comprise of two modules, namely the thermocycling and fluorometric module and the temperature control and data acquisition module. Figure 3.5 presents the proposed design for the PCR thermocycling and fluorometric module. The focusing distances for the lens are calculated in Appendix A.
Figure 3.5: (a) Front view of proposed PCR thermocycler design and (b) End view of proposed PCR thermocycler design
3.8.2.1 Brief description of components in the PCR thermocycling and fluoremetric module

This module provides the thermocycler with the capabilities to achieve rapid thermocycling as well as real-time fluorescence detection. To achieve a compact thermocycler design, the chief limiting factor is the size of the real-time fluorescence detection system, as the thermocycling components (e.g., heat source, fans) are relatively small. The main components in this module are the IR radiation source, cylindrical lens, IR-transmitting filter, excitation source, excitation wavelength filter, detection wavelength filter and cooling fans. The following briefly describes these components. More detailed description of these components is further presented in Chapter 6.

A possible choice of infrared (IR) source could be halogen tungsten lamp that can radiate thermal energy to the PCR chip. It has been shown by Oda et. al. [26] that tungsten light bulb could be used as the IR source. The IR thermal energy will be transmitted through an IR-transmitting filter. This filter will eliminate wavelengths that could interfere with the PCR reaction, while allowing wavelengths in the IR (greater than 800nm) region to pass through. Filtering also helps to reduce the occurrence of temperature gradients in the sample or partial boiling of the sample. To achieve cooling, cooling fans are used. The module will likely incorporate more than one fan to attain the desired rate of cooling and temperature uniformity. Other preferred characteristics of the fan include low power consumption and lightweight.

To excite the fluorescent dye intercalated in the amplified DNA strands, an excitation light source is required to produce a strong fluorescence signal. The wavelength of this light source is in the visible or near infrared regions, depending on the type of dye used. A band-pass interference filter known as the excitation wavelength filter is needed to allow light in the
desired region of excitation wavelength to be transmitted onto the PCR sample. Hence, light of other wavelengths will be removed from the transmitted light by this filter.

As for fluorescence detection, a *cylindrical lens* will image the emitted fluorescence from the PCR reaction sample onto the fluorescence detection system. In addition, the *detection wavelength filter* is required. It is a band-pass interference filter to allow wavelength of the emitted fluorescence to pass through, depending on the fluorescent dye used. Hence, this can effectively remove light from other interference sources other than from the capillary array to be detected by the fluorescence detection system. In other words, the filter will greatly improve the specificity of the detection of fluorescence emission from the PCR amplified products. PCR fluorescent dyes, such as FAM, Tet, Tamra and ROX have detection wavelengths of 520nm, 550nm, 585, and 628nm respectively [62]. Finally, the *fluorescence detection system* detects the fluorescence emission from successful PCR products in real-time. Common devices for the fluorescence detection are photomultiplier tube (PMT) [93], CCD cameras in many conventional thermocyclers [94], optical scanner developed by MJ Research [94] and photodiodes [62].

**3.8.2.2 Brief description of components of PCR temperature control and data acquisition module**

This module provides the thermocycler with the capabilities to control heating and cooling elements in rapid succession to attain rapid thermocycling. In addition, it allows fast data processing capabilities to enable real-time results monitoring. Hence, the main components in this module are the temperature controller, data acquisition/processing unit and
power supply. The following briefly describes these components. More detailed description of these components is further presented in Chapter 6.

As the name implies, a temperature controller is an instrument used to control temperature. It obtains an input from a temperature sensor and has an output that is connected to the control elements, which are the heat source and fans. The most accurate and stable temperature controllers utilize proportional with integral and derivative control (PID). This type of controller combines proportional control with two additional adjustments, which helps to automatically compensate for changes in the system. These adjustments, integral and derivative, are expressed in time-based units; they are also referred to by their reciprocals, reset and rate, respectively. The proportional, integral and derivative terms must be individually adjusted to a particular system using trial and error. It is best used in systems, which have a relatively small mass, and those, which react quickly to changes in the energy added to the process. The PID control will be discussed in detail in the subsequent section 3.8.4.

The data acquisition/processing unit acquires and converts video signals from the fluorescence detection system into digital signals and transfers them to a PC. It usually utilizes a RS-232 serial port or USB interface for communication to a PC and is capable of high-speed data communications.

Another essential component of this module is the power supply. This is an AC to DC power supply to provide DC power to the cooling fans, as well as any other devices that require DC power. It should be capable of a standard output of +/-5 to 12 Vdc.
3.8.3 **PID Temperature controller**

To achieve the thermal cycling described in Figure 2.7, a high-speed temperature controller is required. Watlow Series 982 ramping controller achieves thermal cycling through PID controls. PID stands for proportional-integral-derivative, which is used to eliminate the "ON/OFF temperature swings" and stabilize the desired temperature. It is able to respond in a few milliseconds after temperature detection, so as to ensure a high degree of sensitivity and allows rapid reaction to temperature fluctuations [101].

The aim of the proportional control is to reduce temperature overshoot. A proportional band is a temperature range set below the desired temperature, as seen in Figure 3.6.

![Proportional Band Diagram](image)

Figure 3.6: (a) Proportional band measured in degrees F and (b) measured as a percent of temperature range [101].

Upon reaching the proportional band, the heat source is periodically switched ON and OFF, thus decreasing the rising temperature.
At the lower portion of the band, the heater is ON most of the time and as the temperature approaches the desired value, the heater is OFF for a longer time. This reduces the temperature overshoot. The heater will switch ON and OFF in an effort to push the temperature back to the desired value. The temperature usually stabilizes at a temperature below the desired temperature. This is called droop. Droop increases with larger proportional bands.

The integral control is to eliminate the droop. It "pushes" actual temperature toward the desired temperature until they agree (see Figure 3.8). This control makes adjustments on the temperature controller by digitally entering a reset value on the controller. The more reset added, the more the control pushes up the actual temperature but it makes the temperature control more unstable.
As the name implies, manual reset is an adjustment made on the temperature controller by the operator. Automatic reset automatically adjusts for set point changes.

The derivative control prevents or minimizes overshoot and undershoot. The derivative control measures the rate of temperature change as seen in Figure 3.9. If the temperature rises too fast, it will begin switching OFF the heater to prevent overshoot. If the temperature is falling too fast, it switches the heater ON longer to prevent undershoot. The control begins before the current temperature reaches the proportional band and it decides that the temperature is rising too fast. As a result, it forces the controller into a proportioning (ON and OFF) action, thus slowing the rate of temperature increase.
In summary, the PID controller can prevent or minimize temperature overshoot or undershoot and stabilizing at the desired temperature. Hence, an accurate and stable temperature control is made possible.

Figure 3.9: Derivative function begins
3.9 Summary

The following table (Table 3.10) describes the specifications for the proposed PCR microchip design.

Table 3.10: Specifications of the proposed design of PCR thermocycler and PCR chip.

<table>
<thead>
<tr>
<th>Specifications</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 PCR chip dimensions</td>
<td>75 mm (length) x 25 mm (width) x 5 mm (depth)</td>
</tr>
<tr>
<td>2 Thermocycling and fluorometric module dimensions</td>
<td>160 mm (length) x 100 mm (width) x 185 mm (height)</td>
</tr>
<tr>
<td>3 Temperature control and data acquisition module</td>
<td>230 mm (length) x 175 mm (width) x 110 mm (height)</td>
</tr>
<tr>
<td>4 PCR reaction chamber</td>
<td>Borosilicate Glass capillary array</td>
</tr>
<tr>
<td>5 Sample volume</td>
<td>1 to 12 μl (depending on type of capillary used)</td>
</tr>
<tr>
<td>6 Temperature sensing device</td>
<td>Thermocouple type “K”</td>
</tr>
<tr>
<td>7 Heating mechanism</td>
<td>Heat radiation from a cylindrical halogen light bulb</td>
</tr>
<tr>
<td>8 Cooling mechanism</td>
<td>Forced convection from in-built miniature cooling fan</td>
</tr>
</tbody>
</table>
Chapter 4

HEAT TRANSFER SIMULATION OF IR HEATING IN
DENATURATION AND EXTENSION PROCESSES

4.1 Introduction

Computer simulations by a commercial analysis software package could be used to virtually simulate a complete range of thermocycler and chip behavior before committing to expensive and time-consuming fabrication and experimental plans.

Nowadays, commercial analysis packages are Window-based, thus making them easily accessible. These not only provide a high degree of familiarity for the majority of users, but also ensure that the softwares are able to bring powerful digital simulation directly to the engineer's desktop easily and affordably.

The commercial software FEMAP TMG, version 6.1 was used in the virtual simulation of the heating phases during the PCR experiment. FEMAP TMG is a standalone Pre- and Post-Processing for engineering finite element analysis. It provides advanced thermal simulation integrated within the modeling environment and a complete analysis solution for conduction, convection, fluid flow, and radiation for both transient and steady state thermal simulations. The main advantage for the use of FEMAP TMG in the simulation of radiation heat transfer during denaturation and extension processes is that it provides capabilities for modeling a wide range of radiative effects, including multiple enclosures, specular and transmissive surfaces, solar or high temperature sources, orbital heating, articulating systems, and temperature-dependent emissivity. In addition, the solution of the radiative exchange problem is based on a radiosity formulation, using a combination of hemicube and ray-tracing techniques to compute the direct view factors. [87]

Since radiation is a heat transfer mechanism that requires the target area to be "visible" to the heat source, the heat source is therefore positioned directly above the capillary array in the thermocycler design to offer the maximum radiation heat transfer. In addition, the distance between the PCR chambers and the heat source should be minimized, so as to enhance the rate of heating rate. However,
sufficient distance between the capillaries and heat source has to be considered for the placement of the IR transmitting lens.

The heat source used in the thermocycler design is a cylindrical halogen light bulb of effective length of 100mm and a width of 10mm. As for the design of the PCR chip, the length of the IR transmitting lens limits the length of the capillary array. The size for the lens is 50mm long x 25mm wide x 5mm thick, thus the effective area of the PCR capillary array is less than 50mm long by 10mm wide. The PCR chip design also plays an important role in the rate of heating and the temperature uniformity within the capillaries and the capillary array. In this case, the effects of capillary internal diameters, capillary wall thickness, intermediate spacing between adjacent capillaries on thermal uniformity and cooling rate are investigated.

4.2 3-D model and meshing in FEMAP TMG analysis package

As seen in Figure 4.1, the components in the thermocycling chamber (in Figure 3.6) are modeled with a long cylinder, a rectangular block and an array of short length capillaries. Fans are not modeled here, as they are not required to achieve heating. The long cylinder simulates the cylindrical radiation heat source and the rectangular block is the infrared (IR) transmitting lens. Dimensions and distances correspond to the actual prototype. Meshes of these components are presented in Figure 4.2, with both the cylindrical heat source and filter more spatially meshed (element size of 0.01m) as compared to the capillary array. The area of interest in this model, of course, is the capillary array and the liquid segments contained within the capillaries. Both the capillaries and the liquid segments are more finely meshed with element size of 0.001m, as seen in Figures 4.3 to 4.6.
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Figure 4.1: model of experiment setup

Figure 4.2: meshed setup (cylindrical heat source, filter and capillary array)
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Figure 4.3: View of entire array of capillaries (meshed)

Figure 4.4: A close-up view of the meshed capillaries. (Shown here is left half of array since array is asymmetric in geometry)
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The liquid segment is modeled within the capillaries. The following figures show the array of liquid segment.

Figure 4.5: cross-sectional view of a single capillary, containing a liquid segment

Figure 4.6: A close-up view of the meshed liquid segments. (Shown here is left region of array since array is asymmetric in geometry)

By default, FEMAP is unit-less. Therefore, all dimensions must be kept consistent with the unit system when defining material properties. Conduction between the surfaces of the capillaries and liquid segment is modeled using a conductive thermal coupling. The surfaces within the model radiate to the Space Enclosure;
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the equivalent of a large cubic surrounding that effectively allows modeling of radiation to the environment.

The material surfaces of the lens, capillary array and the liquid segment are defined as specular and transmissive surfaces and their respective thermo-optical properties stated in Table 4.1 are inputted into the software. Water properties are used for the material properties of the liquid segment contained within the capillaries. Surface geometry for radiation models is defined using Plate elements created directly on part surfaces. For accurate modeling of focusing effects, curved surface elements can be used.

Table 4.1: Table of thermo-optical properties and material properties

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflectivity, $\rho$</td>
<td>? 0.04</td>
<td>0.10</td>
<td>? 0.04</td>
<td>- *</td>
<td>? 0.003</td>
</tr>
<tr>
<td>Transmissivity, $t$</td>
<td>0.92</td>
<td>Opaque</td>
<td>0.92</td>
<td>- *</td>
<td>0.9</td>
</tr>
<tr>
<td>Emissivity, $e$</td>
<td>0.90</td>
<td>0.96</td>
<td>0.90</td>
<td>0.90</td>
<td>0.96</td>
</tr>
<tr>
<td>Density (kg/m$^3$)</td>
<td>2230</td>
<td>2702</td>
<td>2700</td>
<td>2500</td>
<td>963.2</td>
</tr>
<tr>
<td>Thermal conductivity (W/m.K)</td>
<td>1.2</td>
<td>2.37</td>
<td>0.76</td>
<td>1.4</td>
<td>0.678</td>
</tr>
<tr>
<td>Specific heat capacity (kJ/kg.K)</td>
<td>0.835</td>
<td>0.903</td>
<td>0.84</td>
<td>0.75</td>
<td>4.204</td>
</tr>
</tbody>
</table>

*Since the cylindrical light bulb is the heat source, these radiation properties are not required.

4.3 IR heating simulations in denaturation process

4.3.1 Boundary and initial conditions

A transient thermal analysis is performed for the denaturation process using FEMAP TMG. Initial temperatures for the liquid segments are at 70°C. A boundary condition of 351W power output is defined on the cylindrical heat source. This is derived from [49], which states that 70.3% of 500W bulb will be
radiated. Both the lens and capillary array are exposed to direct radiative heating from the cylindrical heat source.

### 4.3.2 Results of IR heating simulations in denaturation process

Figures 4.7 below presents the simulation results from half of the capillary array are presented since the geometry of the array is asymmetric. The numbers in white in Figure 4.7 identify the liquid segments in their respective capillaries in the asymmetric half of the capillary array.

![Temperature distribution on the liquid segment (top view) at 2.5 seconds.](image)

As seen in Figures 4.7, the liquid segments attained about 95.5°C to 95.9°C in about 2.5 seconds. The figure also indicates a uniform temperature distribution on the liquid segments, especially from the fifth segment from left onwards. The largest temperature difference is approximately 0.4°C.
For better understanding of the temperature ramping rate of water segments, analysis results from simulations are compiled and plotted in Figure 4.8 below,

![Figure 4.8: History plot of center of water segments. Only results from the asymmetric half of the capillary array are plotted here.](image)

It can be seen that the capillaries are heated from 70°C to 95°C in approximately 2.5 seconds. It is noted that the ramping rate of all capillaries is almost equivalent, which is due to uniform heating. This rate of heat ramping is approximately 10°C/s.

Subsequent Figures 4.9 to 4.11 are variation plots of temperature distribution of the water segments with respect to different positions along x-axis (at y=0) and y-axis at time = 1, 2 and 2.5 seconds respectively.
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Figure 4.9: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Time = 1s.

Figure 4.10: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Time = 2s.
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Figure 4.11: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Time = 2.5s.

From Figure 4.9 to 4.11, we see that the temperature difference along x-axis (at y=0) of the capillary array is very small for the different time frames. The largest temperature variation is slightly more than 0.3°C. Temperature distribution within the water segment is also very uniform with the largest difference of about 0.02°C. This occurs between the center and edge of the water segment. Hence, the possibility of achieving uniform and rapid heating by radiation heat transfer is clearly shown in this analysis.
Figure 4.12: Comparison of temperature ramping rate of water segment and capillary. Only results from capillary 1 (left graph) and 12 (right graph) are plotted here.

In addition, from Figure 4.12, the rate of heating of the glass capillary and water segment is approximately equivalent. For both capillary 1 and 12, the temperature difference between the glass capillary and water is about 0.002°C, with the glass capillary at a slightly lower temperature. This is likely due to the high transmissivity of borosilicate glass material of the capillary, which allows most of the radiated heat in the form of infrared waves to be transmitted to the water segment, thus water segment is heated faster. The capillary, in turn, is heated up by both heat radiation from the heat source as well as heat conduction from the water segment. Since the thermal mass of the capillary (10mm long and 0.1mm thick) is very small, the heating rate of the capillary is also comparable to that of the water segment.

4.4 IR heating simulations in extension process

4.4.1 Boundary and initial conditions

A transient thermal analysis is performed for the extension process using FEMAP TMG. This simulation is similar to the denaturation process. Both the lens and capillary array are exposed to direct radiative heating from the cylindrical heat
source. However, the initial temperatures for the liquid segments are at 55°C while the boundary condition for the cylindrical heat source is still at 351 W.

### 4.4.2 Results from simulation of extension process

Solutions from the simulation are shown in Figures 4.13 and 4.14 below. Only results from half of the array are presented due to the asymmetric geometry of the array. The numbers in white in Figure 4.13 identify the liquid segments in their respective capillary, located in the asymmetric half of the capillary array.

![Figure 4.13: Temperature distribution of the liquid segments (top view) at 1.6 seconds. Numbers in white identify the liquid segments in their respective capillary located in the asymmetric half of the capillary array.](image)

The simulation result is similar to the denaturation process, except that the water segments are heated from 55°C to 70°C. As seen in Figures 4.13, the liquid segments are heated to approximately 70.5°C to 70.9°C in about 1.6 seconds. The figure also indicates a relative uniform temperature distribution on the liquid segments, especially from the fifth segment (from left) onwards. The largest temperature difference is approximately 0.4°C.
Results from simulation are plotted in Figure 4.14 to 4.16 for better understanding and comparison.

![History plot of water segments in capillary array during extension process](image)

Figure 4.14: History plot of liquid segment of elements located at the center of segment

From Figures 4.14, the capillary array heats from 55°C to 70°C in about 1.6 seconds. Uniform heating results in the near equivalent thermal ramping rate of all capillaries. The heat ramping rate is approximately 9.4°C/s. Figure 4.15 and 4.16 are variation plots of temperature distribution of the water segments with respect to different positions along x-axis (at y=0) and y-axis at time = 1 and 1.6 seconds respectively.
Figure 4.15: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Time= 1s.

Figure 4.16: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Time=1.6s.

From Figure 4.15 to 4.16, we see that the temperature difference along x-axis (at y=0) of the water segments in the capillary array is very small. The largest temperature variation is about 0.4°C. Temperature distribution within the water segment is also very uniform with the largest difference of 0.02°C. This occurs between the center and edge of the water segment. Hence, as seen from the above
analysis, uniform and rapid heating by radiation heat transfer during the extension process is possible.

4.5 Investigations of the effect of capillary wall thickness on rate of heating and temperature uniformity

To further understand the effects of capillary wall thickness on heating, variations in capillary wall thickness are made in this investigation. Denaturation process is simulated here as the heating process using FEMAP TMG analysis software.

4.5.1 Boundary and initial conditions

In the analysis, the internal diameter of capillary is a constant of Imm, as seen in Figure 4.17. Hence, the liquid volume is kept constant for the different analysis of varying wall thickness, t. The wall thickness employed in this analysis is equivalent or approximated to the capillary thickness that is readily available commercially. The initial temperature for the liquid segment is at 70°C and the boundary condition for the cylindrical heat source is still at 351W.

Figure 4.17: top view of part of the capillary (left); zoom-in side view of a part of the capillary array (right).
4.5.2 Evaluation of simulation results

Solutions from the simulation are plotted and analyzed in this section. The rate of heat ramping for capillary wall thickness of 0.015mm to 0.2mm is shown in Figure 4.18 below. It is based on results from the center capillary in the array.

![Effect of capillary thickness to rate of heating](image)

**Figure 4.18**: History plot of the effect of capillary wall thickness on heating rate

As seen in Figure 4.18, there is little change to the heating rate for different wall thickness. Time taken to heat the liquid segment from 95°C to 55°C is 2.45 to 2.5 seconds. The reason is likely due to the high transmissivity property of the borosilicate glass material in the IR wavelengths.

Figures 4.19 to 4.22 are variation plots of temperature distribution of capillary array of different capillary thickness with respect to different positions along xaxis (at y=0) and y-axis.
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Figure 4.19: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Capillary wall thickness is 0.015mm. Time = 2.5s.

Figure 4.20: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Capillary wall thickness is 0.05mm. Time = 2.5s.
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Figure 4.21: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Capillary wall thickness is 0.1mm. Time = 2.55s.

Figure 4.22: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Capillary wall thickness is 0.2mm. Time=2.5s.

From Figures 4.19 to 4.22, we see that the temperatures of water segments in capillary array along y-axis and x-axis (at y=0) are uniform. The temperature difference in capillary array is between 0.22 to 0.3°C along x-axis (at y=0), whereas along y-axis, the temperature difference is about 0.001 to 0.005°C between the center and edge of a water segment. By comparison of results in
Figures 4.19 to 4.22, changes to the thickness of capillary wall have little or no effect on the thermal uniformity within a water segment as well as within the capillary array.

From above results, the thickness of glass capillary is found to have little or negligible effect on both the rate of heating and temperature uniformity of the water segments.

4.6 Investigations of the effect of capillary internal diameter on rate of heating and temperature uniformity
Different capillary internal diameters are investigated on their effects on heating rate and temperature uniformity.

4.6.1 Boundary and initial conditions
In this investigation, a constant wall thickness of 0.025mm is used (seen in Figure 4-23), which is also small enough to have negligible or minimal interference on the simulation results. The internal diameter, ID, of capillaries used for this analysis is equivalent or approximated to the size of products available commercially. The initial temperature for the liquid segment is at 70°C and the boundary condition for the cylindrical heat source is still at 351W.
4.6.2 Evaluation of simulation results

Solutions from simulations are tabulated and plotted for evaluation of the effects of capillary internal diameter on rate of heating and temperature uniformity. Figure 4.24 below is a plot of heat ramping rate with respect to time, for different capillary internal diameter. All results plotted in Figure 4.24 are obtained from center capillary in the capillary array.

Figure 4.23: top view of part of the capillary (left); zoom-in side view of a part of the capillary array (right).
Figure 4.24: History plot of the effect of capillary internal diameter on heating rate

From Figure 4.24, for ID of 0.025mm, time taken to heat from 70°C to 95°C is about 2.28 seconds; while for ID of 1mm, time taken is about 2.5 seconds. A smaller ID means a smaller water volume, thus facilitating a faster heating rate. However, the time difference between ID of 0.025mm to 1mm is only about 0.22 seconds, which implies little improvement in the rate of heating.

The following Figures 4.25 to 4.28 are variation plots of temperature distribution of capillary array of different capillary internal diameter (ID) with respect to different positions along x-axis (y=0) and y-axis.
Figure 4.25: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Capillary internal diameter is 0.025mm. Results are at time=2.27 seconds.

Figure 4.26: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Capillary internal diameter is 0.1mm. Results are at time=2.3 seconds.
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Figure 4.27: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Capillary internal diameter is 0.5mm. Results are at time=2.4 seconds.

Figure 4.28: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 6 and 12 (left) and along x-axis (at y=0) for asymmetric half of capillary array (right). Capillary internal diameter is 1mm. Results are at time=2.5 seconds.

From Figures 4.25 to 4.28, we see that the temperatures of water segments in capillary array along y-axis and x-axis (at y=0) are uniform. The temperature difference is between 0.18 to 0.2°C along line x-x, whereas along y-axis, the temperature difference is about 0.001 to 0.005°C between the center and edge of
the water segment. From Figure 4.25 at ID of 0.025mm, we see that capillaries 2 to 5 are at near equivalent temperature whereas capillaries 6 to 12 are at similar temperatures. By comparison of Figure 4.25 to 4.28, temperature difference becomes greater as ID increases. Hence, a smaller capillary ID allows better temperature uniformity along line x-x, except between capillaries 5 and 6, which experienced a greater change in temperature. This improvement in temperature uniformity due to increased ID is not significant as the largest temperature difference at ID of 1mm is only slightly less than 0.2°C, which is still considered uniform.

Since internal diameter of 1mm is one of the larger specifications for glass capillaries, thus, we can conclude that the internal diameter of the capillaries has little significant impact on both the rate of heating and thermal uniformity. Similarly, this is due to the high IR transmissivity property of the borosilicate glass material.

### 4.7 Investigations of the effect of capillary-to-capillary spacing on rate of heating and temperature uniformity

The effect of capillary-to-capillary spacing on heating and temperature uniformity is also an important design considerations and must be examined.

#### 4.7.1 Boundary and initial conditions

In this case, both capillary internal diameter and wall thickness are fixed at values of 1mm and 0.1mm respectively, shown in Figure 4.29. In this investigation, capillary-to-capillary spacing, s, is changed for the analysis on heating rate and temperature uniformity. The initial temperature for the liquid segment is at 70°C and the boundary condition for the cylindrical heat source is still at 351W.