Development of IR-mediated thermocycling system and multi-reaction PCR chip for DNA amplification

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4.7.2 Evaluation of simulation results

Results from simulation on the effects of capillary-to-capillary spacing are evaluated here. Figure 4.30 is a plot of heat ramping rate with respect to time, for different capillary-to-capillary spacing. All results plotted in Figure 4.30 are obtained from the centermost capillary in the capillary array.

Figure 4.30: History plot of the effect of capillary-to-capillary spacing on rate of heating

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From Figure 4.30, time taken for the different capillary-to-capillary spacing to heat from 70°C to 95°C is about 2.5 seconds. Capillary-to-capillary spacing of 0.5mm and 0.8mm allows a heating rate of about 0.02 seconds faster than the rate achieved by a spacing of 0mm, 0.1mm and 0.2mm.

Figures 4.31 to 4.35 are variation plots of temperature distribution of capillary array of different capillary-to-capillary spacing with respect to different positions along x-axis (at $y=0$) and y-axis. Capillary-to-capillary spacing of 0.1mm will allow up to 18 capillaries in the array. Conversely, spacing of 0mm, 0.2mm, 0.5mm and 0.8mm will accommodate 19, 17, 14 and 12 capillaries respectively.

Figure 4.31: 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 to capillary spacing is 0.1mm. Time = 2.5s.

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Figure 4.32: 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 to capillary spacing is 0.2mm. Time = 2.5s.

Figure 4.33: 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 to capillary spacing is 0.5mm. Time=2.5s.

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Figure 4.34: 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 to capillary spacing is 0.8mm. Time = 2.5s.

Figure 4.35: Variation plot of temperature versus position along y-axis for water segments in capillary 1, 9 and 19 (left) and along x-axis (at $y=0$) for asymmetric half of capillary array (right). Capillary to capillary spacing is 0mm. Time=2.6s.

From Figures 4.31 to 4.35, we see that the temperatures of water segments in capillary array along y-axis are uniform, whereas the temperature profile along x-axis (at $y=0$) is not as uniform. The temperature difference varies from about 0.3°C for spacing of 0.8mm to 1°C for spacing of 0.1mm along x-axis (at $y=0$),

whereas along y-axis, the temperature difference is about 0.003 to 0.01 °C between the center and edge of the water segment. Hence, a smaller capillary-to-capillary distance will cause a bigger temperature difference along x-axis. The greater temperature difference due to small capillary-to-capillary spacing could be due to some radiation heat transfer between capillaries that are in close proximity. Since both the water segments and borosilicate glass material have high IR transmissivity, capillaries in the central region of the array will have more heat exchanged through radiation from water segments in neighboring capillaries as compared to capillaries near the ends of the array. This is also the reason that the temperatures at the central region of the array along x-axis (at $y=0$) are more uniform. Capillaries at the ends have fewer capillaries in their vicinity; hence less radiation heat transfer is experienced. In addition, end capillaries are more exposed to the surroundings, which encourage free convection. Therefore, a greater decline in the temperature profile at the end capillaries is resulted. As for within the capillary itself (along y-axis), the temperature profile is found to be relatively uniform. On the other hand, having no capillary-to-capillary spacing allows thermal cross-talk between capillaries, which in turn, can improve the temperature difference along the x-axis to about 0.3 °C.

Hence, from the above results, we see that the capillary-to-capillary spacing has little effect on the rate of heat transfer.

4.8 Summary

For the proposed design of the thermocycler and PCR chip, it can be seen that the transition for capillaries to heat from 70 °C to 95 °C during the beginning of denaturation process takes approximately 2.5 seconds (Figure 4.8). The capillaries are uniformly heated and the rate of heat ramping is approximately 10 °C/s. The largest temperature difference within the array (along x-axis) is about 0.3 °C.

During the transition from annealing process to extension process, the capillary array is heated from 55 °C to 70 °C in about 1.6 seconds (Figure 4.14). Similar to

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the results from denaturation process, uniform heating causes the thermal ramping rate of all capillaries to be almost equivalent. The heat ramping rate is approximately 9.4°C/s .

From the subsequent investigations, it is noted that capillary wall thickness and its internal diameter has little or negligible effect on rate of heating and temperature distribution with the array and within a single capillary. This is due to the high IR transmissivity property of the borosilicate glass material, which allows the water segment contained within the capillary to be heated, without first heating the capillary itself.

However, capillary-to-capillary spacing is found to have a more significant impact on the temperature uniformity within the array (along x-axis). The greatest temperature difference between capillaries can be up to 1°C for a spacing of 0.1mm. This temperature difference can be further reduced with the incorporation of the PID control during the thermocycling process. Therefore, the temperature difference can be improved to be less than 1°C , which in turn downplays the significance of capillary-to-capillary spacing on temperature uniformity. Hence, even though a larger spacing is preferred to achieve a smaller temperature difference, it is not an important criterion during heating in PCR chip design. The capillary-to-capillary spacing also has little effect on the rate of heating. In addition, from the variation plots along x-axis, it can be seen that capillary temperature is similar or approximated to the temperature of the water segments.

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5.1 Introduction

Extensive simulations of the thermocycler and chip behavior are conducted before undergoing time-consuming and costly fabrication process. In Chapter 4, FEMAP TMG was used in the solution of the heating processes, namely denaturation and extension process. Since radiation heating and convective cooling are two different modes of heat transfer, IcePak 4.0 analysis package is utilized to provide an accurate solution of the cooling process during the annealing stage. IcePak is a fully interactive, object-based thermal management computational fluid dynamics (CFD) analysis software. It has a direct CAD interface, and its design integration is made easy by providing faster model import to speed up your thermal analyses. The main advantage for the use of IcePak is its capability to incorporate non-linear fan curves into its analysis. This feature is not available in other analysis packages and it enables IcePak to provide a more realistic fan modeling especially for cooling processes, such as annealing, which are achieved primarily by fans.

The IcePak software solves the governing mass, momentum and energy (Navier-Stokes) equations numerically using the finite volume method. Since no heat loads were applied in the system and the energy equation is uncoupled from the other equations in forced convection, only the mass and momentum equations were solved. For problems dominated by forced convection, IcePak software computes the Reynolds number (Re) and the Peclet number (Pe), both of which are dimensionless. The Reynolds number measures the relative importance of inertial forces and viscous forces. When it is large, inertial forces dominate, boundary layers form, and the flow may become turbulent. The Peclet number is similar to the Reynolds number and measures the relative importance of advection to diffusion for the transport of heat. For most flows simulated by Icepak, both the Reynolds and Peclet numbers are large.

In the thermocycler design, annealing phase is made possible by forced convection with several fans strategically positioned. Hence, to ensure relative

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thermal uniformity and rapid cooling rate, a study of the placement and angle of inclination of fans for the thermocycler design is carried out. Another important factor of consideration in this study will be the volumetric flow-rates of the fans used. Furthermore, the design of PCR chip is an important factor, which could affect both the cooling rate and the temperature uniformity of the PCR reaction chambers and the chamber array. The elements of chip design considered here would be internal capillary diameters, capillary wall thickness and capillaries-to-capillary spacing.

The Icepak software can simulate the capillary surface characteristics by specifying an appropriate surface roughness and thermal conditions such as heat transfer coefficient. Table 5.1 presents the properties of the materials used in both numerical and experimental study, namely borosilicate glass capillaries, ambient air and water sample.

Table 5.1: Property table for different materials

1	Material	Borosilicate glass [49, 68]	Air [49]	Water [40, 49, 85]
2	Density (kg/m ³)	2230	1.1614	963.2
3	Thermal Conductivity (W/m.K)	1.2	0.0439	0.678
4	Specific Heat Capacity (KJ/kg.K)	0.835	1.04	4.204

Fans modeled are circular, internal fans with a manufacturer specified fan characteristic curve. Fans used are Papst 400F series 412FH fan type. The fan characteristic curve in Appendix E was used in the numerical solutions as it describes accurate fan behaviour between pressure drop and airflow. Fan characteristic curves provided in the datasheet are in units of volumetric flow rate (CFM or m³/h) vs. Pressure (Pa). Table 5.2 presents parameters, such as approximated initial and ambient temperatures and fan characteristic curves used during both numerical and experimental study. Heat transfer coefficient is calculated as shown in Appendix D and used in numerical solutions.

Table 5.2: Property table for different materials

1	Initial Temperature (°C)	95
2	Ambient Temperature (°C)	25
3	Fan Characteristic Curve	Characteristic Curve 3 in Appendix E
4	Heat Transfer Coefficient (W/m ² .K)	58

5.2 Investigations of the effects of airflow directed at an angle to the capillary array

The design of the cooling system is limited by the heating system design. As stated in Chapter 4, the cylindrical light bulb (IR radiation source) will be situated above the capillary array to provide a direct and uniform means of radiation heat transfer. In addition, to achieve real time monitoring of DNA amplification, the fluorescence detection system will have to be in a location that can allow all capillaries to be "seen" by the detection system. Hence, the only other feasible position will be situated directly beneath the capillary array.

As a result, the cooling system can only be positioned at Surfaces 1 to 4, shown in Figure 5.1. Since forced convection is utilized in this thermocycler design as the primary heat transfer mechanism for cooling, airflow characteristics experienced by the capillary array are investigated to determine their effects on the rate of cooling and thermal uniformity. Airflows experienced by the capillary array will be characterized as parallel flows and perpendicular flows. Parallel flows are airflows in parallel to the capillaries, whereas perpendicular flows are perpendicular to the capillaries array (see Figure 5.1).

Figure 5.1: Plan view of thermal cycling chamber with fan directions

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Hence, this section will investigate the effects on the thermal uniformity and cooling rate of the capillary array when subjected to parallel flow or perpendicular flow and at different angles.

Figure 5.2: Angle of inclination, θ , for (a) parallel flow and (b) perpendicular flow

5.2.1 Boundary and initial conditions

In this investigation, the capillary array is suspended in an infinite domain. The capillaries are initially at temperatures of 95°C and airflow at temperatures of 25°C is projected onto the array. Parallel airflow is simulated at angles of 0, 30, 60 and 90 degrees to the capillaries and followed by perpendicular airflow also at angles of 0, 30, 60 and 90 degrees to the capillary array. Characteristics of airflow are obtained from the fan curve of Papst Series 405FH fan [89] and these

characteristics remain constant throughout the investigations. In this analysis, all factors will be identical except for the change in flow angles and the type of flow (parallel or perpendicular).

The capillary array will be subjected to only one type of flow at any time during the course of simulations. In the subsequent section, the results due to parallel flows will be presented first and followed by results obtained from perpendicular flows.

5.2.2 Evaluation of the effects of airflow directed at an angle to the capillary array

The effects of airflow directed at an angle to the capillaries are essential preliminary considerations to the thermocycler design. As mentioned before, simulations of fans at different inclination angles are conducted in this analysis. In the section, their results are presented and evaluated.

Firstly, the rate of temperature change of water segments when subjected to airflows at 90° is plotted in Figure 5.3 below.

History plot of water segments in capillary at 90° flow during annealing process

Figure 5.3: History plot of water segments in capillary at 90° flow during annealing process

From Figure 5.3, the water segments cooled from 95°C to 55°C in about 1.65 seconds. The approximated cooling rate is 24.24°C/s . At time 1.65 seconds,

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majority of the capillaries has achieved 55°C and their temperature distributions of water segments along x and y-axis are plotted below.

Figure 5.4: Variation plot along y-axis (left) and variation plot along x-axis (right). Flows are at 90° . Time is at 1.65s.

The above results shown in Figure 5.3 and 5.4 are applicable to both parallel and perpendicular flows. This is because at 90° , both types of airflows are situated at the same location, which is directly above the capillary array.

In Figure 5.4 (left), the temperature distribution of water segments in capillary 1, 6 and 12 are shown. The water segments are located between 0.05 and 0.06 m. The plateau portion of Figure 5.4 (left), between 0.05 and 0.06 m, also represents about 80% of the capillary length. By comparison, we see that the water segment in capillary 12 is at the lowest temperature. This is likely because capillary 12 is positioned near the center of the directed airflow, hence experiencing greater cooling effect as compared to other capillaries in the array. However, by comparing only the plateau portion, temperature uniformity in the water segments (along y-axis) is found to be less than 0.485°C .

On the other hand, the numerous peaks in the Figure 5.4 (right) indicate the temperatures of the water segments; whereas, the temperature drops between each peak define the lower air temperature between the capillaries. The largest temperature difference along x-axis occurred between water segment of capillary 1 and water segment of capillary 12. The difference is about 037°C .

The rate of temperature change of water segments when subjected to parallel flows of 0° is plotted in Figure 5.5 below.

Figure 5.5: History plot when parallel flows are at 0°

As seen in Figure 5.5, the capillaries cool from 95°C to 55°C in 2.45 to 2.5 seconds. The rate of cooling is 16.16°C/s . Similarly, when the bulk of the capillaries have attained 55°C at 2.45 seconds, the temperature distribution of water segments along x and y-axis are plotted below.

Figure 5.6: Variation plot along y-axis (left) and variation plot along x-axis (right). Parallel flows are at 0° . Time is at 2.45s.

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In Figure 5.6 (left), the temperature distribution of water segments in capillary 1, 6 and 12 are shown. The water segments are located between 0.05 and 0.06 m. The plateau portion of Figure 5.6 (left), between 0.05 and 0.06 m, also represents about 80% of the capillary length. By comparison, we see that the water segment in capillary 1 is at the lowest temperature. However, by comparing only the plateau portion, temperature uniformity in the water segments (along y-axis) is found to be less than 0.94°C .

The numerous peaks in Figure 5.6 (right) represent the temperatures of the capillaries and water segments; whereas, the temperature drops between each peak define the lower air temperature between the capillaries. The largest temperature difference along x-axis occurs between water segment of capillary 1 and water segment of capillary 2. The difference is about 1.35°C .

When the parallel flows are at 30° to the capillaries, the rate of temperature change of water segments is plotted in Figure 5.7.

Figure 5.7: History plot when parallel flow is at 30°

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As seen in Figure 5.7, the capillaries cool from 95 to 55°C in 1.8 seconds. The rate of cooling is 22.22°C/s. At 1.8 seconds, the temperature distribution of water segments along x and y-axis are plotted in Figure 5.8 below.

Figure 5.8: Variation plot along y-axis (left) and variation plot along x-axis (right). Parallel flow is at 30°. Time is at 1.8s.

Figure 5.8 (left) presents the temperature distribution of water segments in capillary 1, 6 and 12. The water segments are located between 0.05 and 0.06 m. The plateau portion of Figure 5.8 (left), between 0.05 and 0.06 m, also represents about 80% of the capillary length. By comparison, we see that the water segment in capillary 12 is at the lowest temperature. However, by comparing only the plateau portion, temperature uniformity in the water segments (along y-axis) is found to be less than 0.59°C.

In Figure 5.8 (right), the numerous peaks represent the temperatures of the capillaries and water segments; whereas, the temperature drops between each peak define the lower air temperature between the capillaries. The largest temperature difference along x-axis occurs between water segment of capillary 1 and water segment of capillary 12, which is about 1.4°C.

The rate of temperature change of water segments subjected to 60° parallel flow is similarly plotted in Figure 5.9 below.

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Figure 5.9: History plot when parallel flow is at 60°

As seen in Figure 5.9, when the parallel flows are at 60° to the capillaries, the capillaries cool from 95 to 55°C in 2.9 to 3 seconds. The rate of cooling is 13.56°C/s.

The following Figure 5.10 shows the temperature distributions of water segments along x and y-axis when the capillaries approach 55°C at 2.9 seconds.

Figure 5.10: Variation plot along y-axis (left) and variation plot along x-axis (right). Parallel flow is at 60°. The is at 2.9s.

In Figure 5.10 (left), the temperature distribution of water segments in capillary 1, 6 and 12 are shown. The water segments are located between 0.05 and 0.06 m. It

is shown that about 80% of the capillary length is represented by the plateau portion in Figure 5.10 (left). By comparison, we see that the water segment in capillary 12 is at the lowest temperature. However, by comparing only the plateau portion, temperature uniformity in the water segments (along y-axis) is found to be less than 0.5°C .

The numerous peaks in Figure 5.10 (right) represent the temperatures of the capillaries and water segments; whereas, the temperature drops between each peak define the lower air temperature between the capillaries. The largest temperature difference along x-axis occurs between water segment of capillary 1 and water segment of capillary 2. The difference is about 1.56°C .

In Figure 5.11, we can observe the rate of temperature change of water segments when subjected to 0° perpendicular flow.

Figure 5.11: History plot when perpendicular flow is at 0°

As seen in Figure 5.11, when the perpendicular flows are at 0° to the capillaries, the capillaries cool from 95°C to 55°C in 1.4 to 3 seconds. The rate of cooling is 18.18°C/s . Figure 5.12 below presents the temperature distributions of water

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segments along x and y-axis when last of the capillaries approaches 55°C at 3 seconds.

Figure 5.12: Variation plot along y-axis (left) and variation plot along x-axis (right). Perpendicular flow is at 0°. Time is at 3s.

In Figure 5.12 (left), the temperature distribution of water segments in capillary 1, 6 and 12 are shown. The water segments are located between 0.05 and 0.06 m. The plateau portion between 0.05 and 0.06m in Figure 5.10 (left) represents up to 80% of the capillary length. By comparison, we see that the water segment in capillary 1 is at the lowest temperature. This is likely due to the proximity of the end capillaries (such as capillary 1 and 24) to the perpendicular flows. However, by comparing only the plateau portion, temperature uniformity in the water segments (along y-axis) is found to be less than 0.2°C.

The numerous peaks in the Figure 5.10 (right) represent the temperatures of the capillaries and water segments; whereas, the temperature drops between each peak define the lower air temperature between the capillaries. The largest temperature difference along x-axis occurs between water segment of capillary 1 and water segment of capillary 12. There is a big temperature difference of about 19.05°C.

The rate of temperature change of water segments when subjected to 30° perpendicular flow is plotted in Figure 5.13.

Figure 5.13: History plot when perpendicular flow is at 30°

As seen in Figure 5.13, when the perpendicular flows are at 30° to the capillaries, the capillaries cool from 95°C to 55°C in 1.8 to 1.85 seconds. The rate of cooling is 21.92°C/s. Figure 5.14 below presents the temperature distributions of water segments along x and y-axis when the capillaries approach 55°C at 1.8 seconds.

Figure 5.14: Variation plot along y-axis (left) and variation plot along x-axis (right). Perpendicular flow is at 30°. Time is at 1.8s.

In Figure 5.14 (left), the temperature distribution of water segments in capillary 1, 6 and 12 are shown. The water segments are located between 0.05 and 0.06 m. The plateau portion between 0.05 and 0.06m in Figure 5.14 (left) represents up to 80% of the capillary length. By comparison, we see that the water segment in capillary- 12 is at the lowest temperature. However, at the plateau portion, temperature uniformity in the water segments (along y-axis) is found to be less than 0.4°C

capillaries and water segments; whereas, the temperature drops between each peak define the lower air temperature between the capillaries. The largest temperature difference along x-axis occurs between water segment of capillary 1 and water segment of capillary 12. The temperature difference is about 1.52°C .

In Figure 5.15, we can observe the rate of change of temperatures in the water segments when subjected to 60° perpendicular flow.

Figure 5.1 5: History plot when perpendicular flow is at 60°

At 60° perpendicular flows, the capillaries cool from 95°C to 55°C in 1.63 to 1.7s. The rate of cooling is 24.02°C/s . Figure 5.16 below presents the temperature

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distribution of water segments along x and y-axis when the capillaries approach
55°C at 1.63 seconds.

Figure 5.16: Variation plot along y-axis (left) and variation plot along x-axis (right). Perpendicular flow is at 60°. Time is at 1.63s.

In Figure 5.16 (left), the temperature distribution of water segments in capillary 1, 6 and 12 are shown. The water segments are located between 0.05 and 0.06 m. The plateau portion between 0.05 and 0.06m in Figure 5.16 (left) represents up to 80% of the capillary length. By comparison, we see that the water segment in capillary 1 is at the lowest temperature. However, at the plateau portion, temperature uniformity in the water segments (along y-axis) is found to be less than 0.5°C.

The numerous peaks in Figure 5.16 (right) represent the temperatures of the capillaries and water segments; whereas, the temperature drops between each peak define the lower air temperature between the capillaries. The largest temperature difference along x-axis occurs between water segment of capillary 1 and water segment of capillary 12. The difference is about 1.45°C.

Table 5.3 presents a summary of all results in Figure 5.3 to 5.16.

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Table 5.3: Comparison of the effects of different angle of airflow on cooling rate and temperature uniformity

Angle of flow	Time taken (s)	Average time taken (s)	Approx. cooling rate ($^{\circ}\text{C/s}$)	Largest temperature differences along x-axis ($^{\circ}\text{C}$)	Largest temperature differences along y-axis ($^{\circ}\text{C}$) within a capillary
0° (parallel)	2.45 - 2.5	2.475	16.16	1.35	0.94
30° (parallel)	1.8	1.8	22.22	1.4	0.59
60° (parallel)	2.9 - 3	2.95	13.56	1.56	0.5
0° (perpendicular)	1.4 - 3	2.2	18.18	19.05	0.2
30° (perpendicular)	1.8 - 1.85	1.825	21.92	1.52	0.4
60° (perpendicular)	1.63 - 1.7	1.665	24.02	1.45	0.5
90°	1.65	1.65	24.24	0.87	0.485

As seen in Table 5.3, the best temperature uniformity and rate of cooling can be achieved by subjecting the capillary array to airflows at 90° to the array. However, the infra-red (IR) heat source is to be positioned directly above the capillary array to enable the best thermal uniformity and desired heating rate. The fluorescence detection system will be directly below the capillary array for optimum detection capabilities. Hence, these design constraints prevent the miniature fans to be positioned at 90° to the array. Instead, a suitable alternative is to have parallel flows at 0° . These flows are capable of attaining good temperature uniformity across the array while maintaining relatively good uniformity of less than 1°C within the water segments. It is also capable of a moderate rate of heating. Another advantage in this type of flows is the ease to achieve flows at 0° . Flows at different angles of inclination are prone to erroneous angular positioning due to positioning inaccuracies. This will lead to non-uniform cooling, which ultimately causes poor temperature uniformity within the capillary array. Flows at 0° allow fans to be in the vertical upright positions for a more compact thermocycler design as compared to fans at an angle of inclination.

In addition, if the exhaust fans are placed parallel to the capillaries as well, the center portion of the capillary array will be cooled much faster due to the flow profile produced by the fans. Hence, the intention for the placement of exhaust

fans at the peripheral is to attain a better temperature uniformity across the capillary array.

5.3 Heat transfer simulation of convective cooling in PCR thermocycling chamber

In the previous section, the capillary array is suspended in an infinite domain. For the current analysis, the PCR thermocycling chamber, as seen in Figure 5.17, involves a rectangular enclosure containing an array of 24 capillaries filled with water segments. Bearing in mind the significance of the placements of intake fans, this chamber design aims to allow all capillaries to be uniformly exposed to the intake fans. Four circular miniature fans are fixed at three sides of the enclosure defining two intake fans on the same side and two exhaust fans at the two opposite sides.

Figure 5.17: Isometric view thermocycling chamber (left); Plan view with fan directions shown (right)

Meshes are generated and refined as shown in Figure 5.18 and 5.19. It can be seen that the area around and at the capillary array and the liquid segments are more finely meshed.

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Figure 5.18: Plan view of mesh of thermocycling chamber

Figure 5.19: Isometric view of entire array of meshed capillaries (left); Isometric view of entire array of meshed water segments (right)

5.3.1 Boundary and initial conditions

A transient thermal analysis is performed using ICEPAK 4.0. The initial condition for the water segments is at 95°C. The fan characteristics, fan curves and physical dimensions used in this simulation are based on the Papst Series 405FH fan [89]. Intake ambient air temperature is fixed at room temperature of 25°C.

5.3.2 Evaluation of simulation results of PCR thermocycling chamber

Results from the simulation are shown in Figures 5.20 and 5.21. The numbers in white in Figure 5.20 are numbers allocated for each individual capillary.

Figure 5.20: Temperature contours of the liquid segments (top view) at 2.2 seconds. Numbers in white are numbers identifying individual capillary

Figure 5.21: Temperature contours of capillaries and surrounding air (top view) at 2.2 seconds. White arrows indicate the airflow direction.

To understand the rate of cooling of the thermocycling chamber design, we can observe the rate of temperature change shown in Figure 5.22.

Figure 5.22: History plot of water segments

From Figure 5.22, the capillaries cool from 95 to 55°C in 2 to 2.5 seconds. In addition, the rate of cooling is approximately 16°C/s. In addition, the temperature distributions with regard to position on the x-axis (at $y=0.035\text{m}$) and y-axis are illustrated in Figure 5.23.

Figure 5.23: Variation plot of temperature versus position on y-axis (left) and x-axis (right).
Time = 2.2 seconds.

Figure 5.23 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located at between 0.03 to 0.04 m. By comparison, we see that the water segments in capillary 7 and 18 are at the lowest temperature. This is likely due to their positions, which are near the center of the intake air streams, hence experiencing a faster cooling rate. In addition, since one end of each capillary is nearer to the intake fan, this capillary end is of a lower temperature than that of the other end. At the plateau portion of Figure 5.23 (left) between 0.03 to 0.04 m, the largest temperature difference within the capillary is found to be approximately 1.5°C.

As explained before, the numerous peaks in the Figure 5.23 (right) represents the temperatures of the capillaries and water segments; whereas, the temperature drops between each peak denote the lower air temperature between the capillaries. The largest temperature difference along x-axis (at $y=0.035\text{m}$) occurred between water segment of capillary 7 and water segment of capillary 12. The difference is about 4.2°C.

5.3.3 Heat transfer analysis of cooling process with some PID control

As seen in Figure 5.23, the largest temperature difference is about 4.2°C, which is not desirable. Hence, PID control has to be incorporated to further enhance the overall thermal uniformity of the capillary array. Since it will be difficult to fully simulate the PID controller with analysis software, it is however possible to simulate certain elements of the PID control.

As explained in Chapter 3, during the preliminary stages of PID control, the fans are switched off when the threshold level above the set temperature is reached. Therefore, in the following simulation, a solution is initially executed with fans activated. The simulation will solve for a period of 1 second, which is the time in which the temperature of most capillaries have attained the preset threshold level. Thereafter, the fans are deactivated and the simulation is restarted again based on end results from the first simulation. The restarted solution will execute for a longer period of 5 seconds.

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The results from the simulation are shown in Figure 5.24 below.

Figure 5.24: Temperature contours of the liquid segments (top view) at 3.37 seconds. Numbers in white are numbers identifying individual capillary.

Even though fans are deactivated in the final phase of the simulation, there is still some residual flow within the enclosure, as seen in Figure 5.25.

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Figure 5.25: Temperature contours of capillaries and surrounding air (top view) at 3.37 seconds. Arrows in white depicts direction of airflow.

Figure 5.26 presents the change of temperature of the water segments with respect to time.

Figure 5.26: History plot of water segments

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From Figure 5.26, capillaries cool from 95°C to 55°C in about 3.3 to 3.4 seconds. With only a preliminary stage of PID control, we can observe that the temperature uniformity of the capillaries is significantly improved. However, the rate of cooling is compromised and is slower when compared to previous analysis without PID control. The cooling rateⁱ is approximately 12°C/s. As a result, thermocycling by proper PID control enables a more uniform temperature distribution within the capillary array as well as a moderately fast cooling rate.

The temperature distribution with regard to position on the x-axis (at $y=0.035\text{m}$) and y-axis for different time frames are plotted in Figure 5.27 to 5.29.

Figure 5.27: Variation plot of temperature versus position on y-axis (left) and x-axis (right). Time = 1 second.

Figure 5.28: Variation plot of temperature versus position on y-axis (left) and x-axis (right). Time = 2 seconds.

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Figure 5.29: Variation plot of temperature versus position on y-axis (left) and x-axis (right). Time = 3.37 seconds.

Figure 5.27 to 5.29 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located at a distance of 0.03 to 0.04m at time = 1s, 2s and 3.37s respectively. At positions of 0 to 0.03m, the air in this region is at relatively higher temperature due to the lack of cooled air supplied when the fans of switched off. However, air at positions of 0.03 to 0.07m experiences a much lower temperature due to the residual intake airflow from the ambient surroundings. In Figure 5.27 to 5.29 (right), the peaks in the graph describe the higher temperatures of capillaries and water segments. We can observe that the temperature distribution of the capillary array along x-axis has improved significantly. The largest temperature difference along x-axis occurred between water segment of capillary 2 and water segment of capillary 9 (seen in Figure 5.27) and this difference is less than 2°C. Temperature difference along y-axis, (the plateau portion of Figure 5.27 to 5.29 (left) between 0.03 to 0.04m), is found to be approximately less than 15°C. Thus, temperatures of water segments in different capillaries are at relatively uniform. Hence, PID control is an essential tool to accomplish fast and accurate PCR thermocycling.

5.4 Investigations of the effects of fan volumetric flow-rate on cooling rate and thermal uniformity

The effects of fan volumetric flow-rate on cooling rate and thermal uniformity are important considerations to the thermocycler design. Simulations of fans of different flow-rates are used in this analysis. Since the process to simulate PID control is tedious and time consuming, the solutions of the various flow-rates are conducted without PID control. Figure 5.30 defines the x-axis and y-axis orientations.

Figure 5.30: 3-D model of capillary array with x-axis and y-axis specified. Arrows in white denote the fan orientation.

5.4.1 Boundary and initial conditions

In the earlier section 5.2, the fan used is Papst Series 405FH fan [89]. Other fans used in the simulations of this section are capable of achieving $0.1 \text{ m}^3/\text{min}$, $0.1333 \text{ m}^3/\text{min}$ and $0.15 \text{ m}^3/\text{min}$ flow-rates. These flow-rates are approximated or equivalent to the flow-rates of fans that are commercially available and small enough to be integrated in the PCR thermocycler [75, 76, 89]. The fan curves of volumetric flow-rates against static pressure are given by the fan datasheets [75, 76, 89] and the ICEPAK software uses them for more realistic fan performance. The initial temperature for the water segments is at 95°C . Intake ambient air temperature is fixed at room temperature of 25°C .

5.4.2 Evaluation of simulation results

Solutions of this analysis are presented in this section. Firstly, Figure 5.31 below presents the change of temperature of the water segments with respect to time for fan volumetric flow rate of $0.1\text{m}^3/\text{min}$.

Figure 5.31: History plot at flow rate of $0.1\text{m}^3/\text{min}$

As seen in Figure 5.31, the fan of $0.1\text{m}^3/\text{min}$ volumetric flow rate cools capillaries from 95°C to 55°C in 2.25 to 2.75 seconds. The rate of cooling is 16°C/s . The temperature distribution, along x-axis (at $y=0.035\text{m}$) and y-axis at time 2.5 seconds are shown in Figure 5.32.

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Figure 5.32: Variation plot along y-axis (left) and variation plot along x-axis (right). Flow rate is $0.1\text{m}^3/\text{min}$. Time = 2.5s .

Figure 5.32 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.32 (left), between 0.03 to 0.04 m, represents the bulk of the capillary length. Similarly, the peaks seen in the Figure 5.32 (right) describe the higher temperatures of capillaries and water segments, whereas the temperature drops between peaks depict the temperature of air between capillaries. We see that the largest temperature difference along x-axis is about 5.2°C . The temperature difference along y-axis within each respective capillary is less than 1°C , which is relatively uniform.

The rate of temperature change for fan volumetric flow rate of $0.1333\text{m}^3/\text{min}$ can be seen in Figure 5.33.

Figure 5.33: History plot at flow-rate of $0.1333 \text{ m}^3/\text{min}$.

Fans of $0.1333 \text{ m}^3/\text{min}$ are able to cool the capillaries from 95°C to 55°C in 2 to 2.5 seconds. The rate of cooling is about 17.8°C/s . The temperature distributions along x-axis (at $y=0.035 \text{ m}$) and y-axis at time 2.5 seconds are plotted in Figure 5.34.

Figure 5.34: Variation plot along x-axis (left) and variation plot along y-axis (right). Flow-rate is $0.1333 \text{ m}^3/\text{min}$. Time = 2.5s.

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Figure 5.34 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.34 (left), between 0.03 to 0.04 m, represents about 80% of the capillary length. The peaks seen in the Figure 5.34 (right) describe the temperatures of capillaries and water segments while the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 5.22°C. The temperature difference along y-axis within each capillary (at the plateau portion) is less than 1°C.

Figure 5.35 presents the rate of temperature change for fan volumetric flow rate of 0.15m³/min.

Figure 5.35: History plot at flow-rate of 0.15m³/min

As seen in Figure 5.35, for fan volumetric flow-rate of 0.15m³/min, the time taken for the capillaries to cool from 95°C to 55°C is about 1.9 to 2.4 seconds. The temperature distributions along x-axis (at y=0.035m) and y-axis at time 2.3 seconds are plotted in Figure 5.36.

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Figure 5.36: Variation plot along y-axis (left) and variation plot along x-axis (right). Flow-rate is 0.15m³/min. Time = 2.3s.

Figure 5.36 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.36 (left), between 0.03 to 0.04 m, represents 80% of the capillary length. The peaks seen in the Figure 5.36 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 5.37°C. The temperature difference along y-axis within each capillary (at the plateau portion) is found to be less than 1°C, hence resulted in a uniform temperature distribution within the capillary. Table 5.4 summarizes the results from Figures 5.31 to 5.36.

Table 5.4: Comparison of the effects of volumetric flow-rate on cooling rate and temperature uniformity

Vol. flow-rate (m ³ /min)	Time taken (s)	Average time taken (s)	Approx. cooling rate (°C/s)	Largest temperature differences along x- axis (°C)	Largest temperature differences along y-axis (°C)
0.1	2.25 – 2.8	2.53	16	5.22	1
0.1333	2 – 2.5	2.25	17.8	5.37	1
0.15	1.9 – 2.4	2.15	18.6	5.4	1

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We can observe that a higher volumetric flow-rate would improve the cooling rate of the capillaries but would result in poorer thermal uniformity in the capillary array. Conversely, a lower volumetric flow-rate would allow better thermal uniformity but a slower cooling rate is resulted. However, since the improvement of the thermal uniformity for a slower flow-rate is not very significant (only an improvement of up to 0.18°C), the PID controller could be used primarily to improve the thermal uniformity. Hence, fans with the highest volumetric flow-rate are preferred to allow an improved cooling rate as well as to compensate for the slower rate of cooling during PID control. In this case, Papst Series 405F fan with a volumetric flow-rate of $0.15\text{m}^3/\text{min}$ ($9\text{m}^3/\text{hr}$) is used [89].

5.5 Investigations of the effects of capillary wall thickness on cooling rate and thermal uniformity

The effects of capillary wall thickness on cooling rate and thermal uniformity are important considerations in the design of PCR chip and thermocycler. Simulations are conducted for capillary array of varying wall thickness. The solutions of the various wall thicknesses are conducted without PID control. Figure 5.37 denotes the x and y-axis orientations.

Figure 5.37: 3-D model of capillary array with x-axis and y-axis specified. Arrows in white denote the fan orientation (left) and zoomed-in view of several capillaries (right).

5.5.1 Boundary and initial conditions

In the earlier simulations, the capillary wall used is 0.1mm thick. The wall thicknesses, t (seen in Figure 5.37, right), considered in the following simulations are 0.01mm, 0.05mm, 0.1mm and 0.4mm. These are approximated or equivalent to several standard thicknesses of capillaries [77,91]. The internal diameter of the capillaries is fixed at 1mm. All other factors such as capillary positions, diameter of water segments, length of capillary and non-linear fan curve, are unchanged in this investigation. The initial temperature for the water segments is at 95°C. The fan characteristics, fan curves and physical dimensions used in this simulation are based on the Papst Series 405FH fan [89]. Intake ambient air temperature is fixed at room temperature of 25°C.

5.5.2 Evaluation of simulation results

Solutions of this analysis will be presented and evaluated. Firstly, Figure 5.38 below presents the change of temperature of the water segments with respect to time for wall thickness of 0.01m.

Figure 5.38: History plot for capillary wall thickness of 0.01mm

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The capillaries take about 0.65 to 0.72 seconds to reach 55°C. The cooling rate is approximately at 58.4°C/s. Temperature distributions along x-axis (at $y=0.035\text{m}$) and y-axis (refer to Figure 5.37 for x-axis and y-axis orientations) at time = 0.7 seconds are presented below.

Figure 5.39: Variation plot along y-axis (left) and variation plot along x-axis (right). Capillary wall thickness is 0.01mm. Time = 0.7s.

Figure 5.39 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.39 (left), between 0.03 to 0.04 m, represents approximately 80% of the capillary length. The peaks seen in the Figure 5.39 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 3.8°C. The temperature difference along y-axis within individual capillary (at the plateau portion) is found to be less than 1.6°C.

Figure 5.40 presents the change of temperature of the water segments with respect to time for wall thickness of 0.05m.

Figure 5.40: History plot for capillary wall thickness of 0.05mm

From Figure 5.40, the capillaries take about 1.2 to 1.45 seconds to reach 55°C. The cooling rate is approximately 30.2°C/s. Temperature distributions along x-axis (at $y=0.035\text{m}$) and y-axis (refer to Figure 5.37 for x-axis and y-axis orientations) at time = 1.45 seconds are presented below.

Figure 5.41: Variation plot along y-axis (left) and variation plot along x-axis (right). Capillary wall thickness is 0.05mm. Time = 1.45s.

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Figure 5.41 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.41 (left), between 0.03 to 0.04 in, represents the bulk of the capillary length. The peaks seen in the Figure 5.41 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 5°C. The temperature difference along y-axis within individual capillary (at the plateau portion) is found to be about 1.5°C, which is relatively uniform.

The rate of temperature change of the water segments for wall thickness of 0.1m is shown in Figure 5.42.

Figure 5.42:History plot for capillary wall thickness of 0.1mm

The capillaries take about 2 to 2.45 seconds to reach 55°C. The cooling rate is approximately 18°C/s. Temperature distributions along x-axis (at y=0.035m) and y-axis at time = 2.3 seconds are presented below.

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Figure 5.43: Variation plot along y-axis (left) and variation plot along x-axis (right). Capillary wall thickness is 0.1mm. Time = 2.3s.

Figure 5.43 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 and 0.04 m. The plateau portion of Figure 5.43 (left), between 0.03 and 0.04 m, represents the bulk of the capillary length. The peaks seen in the Figure 5.43 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 5.2°C. The largest temperature difference along y-axis within individual capillary (at the plateau portion) is found to be about 15°C.

The rate of temperature change of the water segments for wall thickness of 0.4m is presented in Figure 5.44.

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Figure 5.44: History plot for capillary wall thickness of 0.4mm

The capillaries take about 8 to 9.8 seconds to reach 55°C. The cooling rate is approximately 4.5°C/s. Hence, temperature distributions along x-axis (at $y=0.035\text{m}$) and y-axis at time = 9.3 seconds are presented below.

Figure 5.45: Variation plot along y-axis (left) and variation plot along x-axis (right). Capillary wall thickness is 0.4mm. Time = 9.3s.

Figure 5.45 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.45 (left), between 0.03 to 0.04 m, represents the bulk of the capillary length. The peaks seen in the Figure 5.45 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. In this simulation, since the wall thickness is increased significantly, the distance between the capillaries is much reduced. This causes the drop in temperatures between capillaries to be less prominent, and thus the downward slopes between peaks (see Figure 5.45, right) is less pronounced as compared to results of smaller thickness. The largest temperature difference along x-axis is about 5.3°C. The temperature difference along y-axis within individual capillary (at the plateau portion of Figure 5.45 (left) between 0.03 to 0.04m) is found to be about 0.1 to 0.2°C, which is very uniform.

The results from the above simulations are summarized in the following Table 5.5 for easy comparison and better understanding.

Table 5.5: Comparison of effects of wall capillary thickness

Wall thickness (mm)	Time taken (s)	Ave. time taken (s)	Approx. cooling rate (°C/s)	Largest temperature difference along x-axis (°C)	Largest temperature difference along y-axis (°C)
0.01	0.65 - 0.72	0.685	58.4	3.8	1.6
0.05	1.2 - 1.45	1.325	30.2	5	1.5
0.1	2 - 2.45	2.225	18	5.2	1.5
0.4	8 - 9.8	8.9	4.5	5.3	0.2

Table 5.5, we see that a thicker capillary will have a slower rate of cooling as well as a less uniform temperature distribution along x-axis. However, the thermal uniformity along y-axis is improved with an increase in capillary wall thickness. Figure 5.46 below shows a plot of the average time taken against the wall thickness.

Figure 5.46: Plot of average time taken vs wall thickness

From Figure 5.46, it could be seen that wall thickness is linearly proportional to the average time taken. Hence, faster cooling corresponds to a smaller wall thickness. For a minimum cooling rate of 10°C/s , the time taken has to be less than 4 seconds. In this case, the wall thickness has to be less than 0.17mm. A cooling rate of 10°C/s is faster compared to the conventional PCR thermocyclers and most PCR systems developed, as seen in Chapter 2. However, several PCR systems, such as the microsystem developed by MATEC [53] and the PCR system of Zou et al [21], have the capability of producing faster cooling rate. Hence, to attain faster thermal cycling, capillaries of wall thickness of 0.1mm or less should be used. The cooling rate of wall thickness of 0.1mm is about 18°C/s .

5.6 Investigations of the effects of internal diameter of capillary on cooling rate and thermal uniformity

The effects of capillary internal diameter on cooling rate and thermal uniformity are also important factors taken into considerations in the PCR chip and thennocycler design. Similarly, simulations are conducted for capillary array of varying internal diameter. These solutions are conducted without PID control.

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Figure 5.47: 3-D model of capillary array with x-axis and y-axis specified with arrows in white denoting the fan orientation (left) and zoomed-in view of several capillaries (right).

5.6.1 Boundary and initial conditions

The capillary wall used is fixed at 0.025mm thick, so as to minimize the effects of wall thickness in interfering with the results in this investigation. The capillary internal diameters, d (seen in Figure 5.47, right) considered in the following simulations are 0.1mm, 0.5mm, 1mm and 1.8mm. These are approximated or equivalent to several standard internal diameters of capillaries [77, 91]. All other factors such as capillary positions, diameter of water segments, length of capillary and non-linear fan curves are unchanged in this investigation. The initial temperature for the water segments is at 95°C. The fan characteristics, fan curves and physical dimensions used in this simulation are based on the Papst Series 405FH fan [89]. Intake ambient air temperature is fixed at room temperature of 25°C.

5.6.2 Evaluation of simulation results

In this section, solutions of this analysis will be presented and evaluated. Figure 5.48 below presents the rate of temperature change of the water segments for internal diameter of 0.1m.

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Figure 5.48: History plot for capillary internal diameter of 0.1mm

The capillaries take about 0.53 to 0.56 seconds to reach 55°C. The cooling rate is approximately 73.4°C/s. The temperature fluctuations along x-axis (at $y=0.035\text{m}$) and y-axis (refer to Figure 5.47 for x-axis and y-axis orientations) at time = 0.55 seconds are presented below.

Figure 5.49: Variation plot along y-axis (left) and variation plot along x-axis (right). Capillary internal diameter is 0.1mm. Time = 0.55 seconds.

Figure 5.49 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.49 (left), between 0.03 to 0.04 m, represents the bulk of the capillary length. The peaks seen in the Figure 5.49 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 5°C. The temperature difference along y-axis within individual capillary (at the plateau portion) is found to be less than 0.5°C, which is a uniform distribution.

Figure 5.50 below presents the rate of temperature change of the water segments for internal diameter of 0.5m.

Figure 5.50: History plot for capillary internal diameter of 0.5mm

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The capillaries take about 0.6 to 0.69 seconds to reach 55°C. The cooling rate is approximately 62°C/s. Temperature distributions along x-axis (at $y=0.035\text{m}$) and y-axis at time = 0.69 seconds are presented in Figure 5.51.

Figure 5.51: Variation plot along y-axis (left) and variation plot along x-axis (right). Capillary internal diameter is 0.5mm. Time = 0.69 seconds.

Figure 5.51 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.51 (left), between 0.03 to 0.04 m, represents 80% of the capillary length. The peaks seen in the Figure 5.51 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 4.2°C. The largest temperature difference along y-axis within individual capillary (at the plateau portion) is found to be 1.5°C.

Figure 5.52 below presents the rate of temperature change of the water segments for internal diameter of 1m.

Figure 5.52: History plot for capillary internal diameter of 1mm

The capillaries take about 0.70 to 0.77 seconds to reach 55°C. The cooling rate is approximately 54.4°C/s. The temperature distributions along x-axis (at $y=0.035\text{m}$) and y-axis at time = 0.77 seconds are presented below.

Figure 5.53: Variation plot along y-axis (left) and variation plot along x-axis (right). Capillary internal diameter is 1mm. Time = 0.77 seconds.

Figure 5.53 (left) presents the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to

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0.04 m. The plateau portion of Figure 5.53 (left), between 0.03 to 0.04 m, represents the bulk of the capillary length. The peaks seen in the Figure 5.53 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 2.9°C. The largest temperature difference along y-axis within individual capillary (at the plateau portion) is found to be about 2°C, thus the temperature distribution is relatively uniform.

Figure 5.54 below presents the rate of temperature change of the water segments for internal diameter of 1.8mm.

Figure 5.54: History plot for capillary internal diameter of 1.8mm.

The capillaries take about 1 to 1.1 seconds to reach 55°C. The cooling rate is approximately 54.4°C/s. The temperature distributions along x-axis (at y=0.035m) and y-axis at time = 1.1 seconds are presented below.

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Figure 5.55: Variation plot along y-axis (left) and variation plot along x-axis ($y=0.035\text{m}$) (right). Capillary internal diameter is 1.8mm. Time = 1.1 seconds.

Figure 5.55 (left) depicts the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 to 0.04 m. The plateau portion of Figure 5.55 (left), between 0.03 to 0.04 m, represents 80% of the capillary length. The peaks seen in the Figure 5.55 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 2.6°C . The largest temperature difference along y-axis within individual capillary (at the plateau portion) is found to be about 2.4°C .

The results from the above simulations are summarized in the following Table 5.6 below.

Table 5.6: Comparison of effects of capillary internal diameter

Internal diameter (mm)	Time taken (s)	Ave. time taken (s)	Approx. cooling rate ($^{\circ}\text{C/s}$)	Largest temperature difference along x-axis ($^{\circ}\text{C}$)	Largest temperature difference along y-axis ($^{\circ}\text{C}$)
0.1	0.53 - 0.56	0.545	73.4	5	0.5
0.5	0.6 - 0.69	0.645	62	4.2	1.5
1	0.7 - 0.77	0.735	54.4	2.9	2
1.8	0.94 - 1.6	1.27	31.5	2.6	2.4

A bigger internal capillary diameter is equivalent to having a bigger water volume. Hence, a bigger internal diameter will cause a slower cooling rate. From Table 5.6, we can also see that a bigger capillary internal diameter will have an improvement in thermal uniformity along x-axis but for temperature distribution along y-axis, it becomes less uniform with a bigger internal diameter.

Figure 5.56: Plot of average time taken vs capillary internal diameter

In Figure 5.56, we see that capillary internal diameter is linearly proportional to the average time taken to cool from 95 to 55°C. However, by comparing with the effects due to capillary wall thickness in Figure 5.46, we see that capillary internal diameter has much less effect on the rate of cooling.

5.7 Investigations of the effects of capillary spacing on cooling rate and thermal uniformity

Convective heat transfer occurs across the surface of the capillaries. Hence, the intermediate spacing between individual capillaries is also an important consideration for PCR chip design. Similarly, simulations are conducted for capillary array of different capillary-to-capillary distance. These solutions are conducted without PID control.

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Figure 5.57: 3-D model of capillary array with x-axis and y-axis specified with arrows in white denoting the fan orientation (left) and zoomed-in view of several capillaries (right).

5.7.1 Boundary and initial conditions

Capillary wall used at a constant of 0.1mm thick. The capillary-to-capillary spacing, s (as seen Figure 5.57, right) used in the following simulations is 0.1mm, 0.2mm, 0.5mm and 0.8mm. These values are based on the desired size of the PCR chip. All other factors such as length of capillary and non-linear fan curve are unchanged in this investigation. The initial temperature for the water segments is at 95°C. Intake ambient air temperature is fixed at room temperature of 25°C. The fan characteristics, fan curves and physical dimensions used in this simulation are based on the Papst Series 405FH fan [89].

5.7.2 Evaluation of simulation results

Results of the simulation of different capillary-to-capillary spacing are compiled and plotted to provide better understanding. Figure 5.58 below shows the rate of temperature change of the water segments when capillary-to-capillary distance is 0.1mm

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Figure 5.58: History plot for capillary-to-capillary spacing of 0.1mm

From Figure 5.58, the capillaries are cooled from 95°C to 55°C in 2.8 to 3.45 seconds. The overall cooling rate is approximated at 12.8°C/s. Figure 5.59 shows the variation plots of the water segments along x-axis (at $y=0.035\text{m}$) and y-axis (refer to Figure 5.57 for x-axis and y-axis orientation) at time = 3.35 seconds.

Figure 5.59: Variation plot for spacing of 0.1mm along y-axis (left) and along x-axis (right). Time = 3.35s.

For a capillary-to-capillary spacing of 0.1mm, the capillary array can accommodate 35 capillaries. In Figure 5.59 (left), the temperature distribution along y-axis of water segments in capillary 1, 8, 18, 26 and 35 are presented and the water segments are located between 0.03 and 0.04 m. The plateau portion of Figure 5.59 (left), between 0.03 and 0.04 m, represents up to 80% of the capillary length. The temperatures of capillaries and water segments describe the peaks seen in Figure 5.59 (right) and the temperature drops between peaks depict the temperature of air between capillaries. The reduction in air gaps between the capillaries causes the temperature drop to be less pronounced as compared to earlier simulations. The largest temperature difference along x-axis is about 3.4°C. The largest temperature difference along y-axis within individual capillary (at the plateau portion) is found to be about 0.3°C.

Following, Figure 5.60 presents the rate of temperature change of the water segments when capillary-to-capillary spacing is 0.2mm.

Figure 5.60: History plot for capillary-to-capillary spacing of 0.2mm

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From Figure 5.60, the capillaries are cooled from 95°C to 55°C in 2.65 to 3.2 seconds. The overall cooling rate is approximated at 13.7°C/s. Figure 5.61 shows the variation plots of the water segments at 0.2mm capillary-to-capillary spacing along x-axis (at $y=0.035\text{m}$) and y-axis (refer to Figure 5.57 for x-axis and y-axis orientation) at time = 3.1 seconds.

Figure 5.61: Variation plot for spacing of 0.2mm along y-axis (left) and along x-axis (right). Time=3.1s.

For a capillary-to-capillary spacing of 0.2mm, 33 capillaries can be accommodated in the capillary array. In Figure 5.61 (left), the temperature distribution of water segments in capillary 1, 8, 17, 25 and 33 are presented and the water segments are located between 0.03 and 0.04 m. The plateau portion of Figure 5.61 (left), between 0.03 and 0.04 m, represents 80% of the capillary length. The numerous peaks seen in the Figure 5.61 (right) describe the temperatures of capillaries and water segments while the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 4.2°C. The largest temperature difference along y-axis within individual capillary (at the plateau portion) is found to be about 0.4°C.

Figure 5.62 presents the rate of temperature change of the water segments when capillary-to-capillary spacing is 0.5mm.

Figure 5.62: History plot for capillary-to-capillary spacing of 0.5mm

From Figure 5.62, the capillaries are cooled from 95°C to 55°C in 2.1 to 2.4 seconds. The overall cooling rate is approximated at 17.8°C/s. Figure 5.63 shows the temperature distributions of the water segments at 0.5mm capillary-to-capillary spacing along x-axis (at y=0.035m) and y-axis (refer to Figure 5.57 for x-axis and y-axis orientation) at time = 2.43 seconds.

Figure 5.63: Variation plot for spacing of 0.5mm along y-axis (left) and along x-axis (right). Time = 2.43s.

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For a capillary-to-capillary spacing of 0.5mm, 27 capillaries are accommodated in the capillary array. In Figure 5.63 (left), the temperature distribution of water segments in capillary 1, 7, 14, 21 and 27 are presented and the water segments are located between 0.03 and 0.04 m. The plateau portion of Figure 5.63 (left), between 0.03 and 0.04 m, represents 80% of the capillary length. The peaks seen in the Figure 5.63 (right) describe the temperatures of capillaries and water segments whereas the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 4.3°C. The largest temperature difference along y-axis within individual capillary (at the plateau portion) is found to be about 0.4°C.

Subsequently, we can observe the rate of temperature change of the water segments when capillary-to-capillary spacing is 0.8mm in Figure 5.64.

History plot of water segments in capillary array at spacing of 0.8mm during annealing process

Figure 5.64: History plot for capillary-to-capillary spacing of 0.8mm

From Figure 5.64, the capillaries are cooled from 95°C to 55°C in 2 to 2.45 seconds. The overall cooling rate is approximated at 18°C/s. Figure 5.65 shows the temperature distributions of the water segments at 0.8mm capillary-to-

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capillary spacing along x-axis (at $y=0.035\text{m}$) and y-axis (refer to Figure 5.57 for x-axis and y-axis orientation) at time = 2.2 seconds.

Figure 5.65: Variation plots for spacing of 0.8mm along y-axis (left) and along x-axis (right). Time=2.2s.

For a capillary-to-capillary spacing of 0.8mm, 24 capillaries can be accommodated in the capillary array. In Figure 5.65 (left), the temperature distribution of water segments in capillary 1, 6, 12, 18 and 24 and the water segments are located between 0.03 and 0.04 m. The plateau portion of Figure 5.65 (left), between 0.03 and 0.04 m, represents the bulk of the capillary length. The peaks seen in the Figure 5.65 (right) describe the temperatures of capillaries and water segments and the temperature drops between peaks depict the temperature of air between capillaries. The largest temperature difference along x-axis is about 5.2°C . The largest temperature difference along y-axis within individual capillary is found to be about 1.5°C .

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Lastly, we have the rate of temperature change of the water segments when there is no capillary-to-capillary spacing in Figure 5.66.

History plot of capillary array at no spacing during annealing process

Figure 5.66: History plot for capillary array with no spacing

From Figure 5.66, the capillaries are cooled from 95°C to 55°C in 0.78 to 1.72 seconds. The overall cooling rate is approximated at 32°C/s. Figure 5.67 shows the temperature distribution of the water segments at no capillary-to-capillary spacing along x-axis (at $y=0.035\text{m}$) and y-axis (refer to Figure 5.57 for x-axis and y-axis orientation) at time = 1.7 seconds.

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Figure 5.67: Variation plots for no spacing along y-axis (left) and along x-axis (right). Time = 1.7s.

For a capillary-to-capillary spacing of 0mm, 37 capillaries can be accommodated in the capillary array. In Figure 5.67 (left), the temperature distribution of water segments in capillary 1, 9, 19, 28 and 37 are presented and the water segments are located between 0.03 and 0.04 m. The plateau portion of Figure 5.67 (left), between 0.03 and 0.04 m, represents the bulk of the capillary length. The peaks seen in the Figure 5.67 (right) define the temperatures of capillaries and water segments. Since there are no air gaps between capillaries, the temperature drops between peaks describe the thermal cross-talk between connecting capillary walls. The largest temperature difference at the "plateau portion" along y-axis is about 3°C. The largest temperature difference along x-axis within individual capillary is found to be about 10°C.

The results from the above simulations are summarized in the following Table 5.7.

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Table 5.7: Comparison of effects of capillary-to-capillary spacing

Capillary-to-capillary spacing (mm)	Time taken (s)	Ave. time taken (s)	Approx. cooling rate ($^{\circ}\text{C/s}$)	Largest temperature difference along line x-x ($^{\circ}\text{C}$)	Largest temperature difference along line y-y ($^{\circ}\text{C}$)
0	0.78 – 1.72	1.25	32	10	3
0.1	2.8 – 3.45	3.125	12.8	3.4	0.3
0.2	2.65 – 3.2	2.925	13.7	4.2	0.4
0.5	2.1 – 2.4	2.25	17.8	4.3	0.4
0.8	2 – 2.45	2.225	18	5.2	1.5

From Table 5.7, we see that smaller capillary-to-capillary spacing allows better temperature uniformity at slower cooling rates. However, with no air gaps, cooling rate is much improved, at the expense of thermal uniformity. This is likely due to the absence of air gaps between capillaries to prevent thermal cross-talk with adjacent capillaries. By comparison, a smaller capillary-to-capillary spacing will have a better thermal uniformity along x-axis and along y-axis. However, as a result of a reduction in spacing, the rate of cooling is slower. Hence, capillary-to-capillary spacing of less than 0.5mm is preferred as there is a significant improvement in temperature distributions.

5.8 Summary

In this chapter, CFD analysis is carried out to provide better understanding of the effects of many important factors of considerations during the design of PCR thermocycler and PCR chip when performing annealing process. The placement of fans is critical in determining a uniform and rapid cooling process. By comparison, the arrangement of fans to provide parallel flow at 0° to the capillary array produces good thermal uniformity (see Figure 5.6) and relatively fast cooling. Under this proposed design for the thermocycler, the PCR chip can be cooled from 95°C to 55°C in about 2.45 seconds (Figure 5.22). The capillaries are relatively cooled to uniform temperature and the rate of cooling is approximately 18°C/s . The largest temperature difference within the array (along x-axis) is about 5.2°C and within a capillary (along y-axis) is about 1.5°C .

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In addition, if the exhaust fans are placed parallel to the capillaries as well, the center portion of the capillary array will be cooled faster due to the flow profile produced by the fans. Hence, the intention for the placement of exhaust fans at the peripheral is to attain better temperature uniformity across the capillary array.

From Figure 5.27 and 5.29, by imitating the behavior of a little PID control in the simulations, the thermal uniformity of the PCR chip can be much improved to less than 2°C along the x-axis and less than 1.5°C along y-axis. Hence, with proper PID control, the droop (see Figure 3.10) of the temperature fluctuations could be eliminated or reduced, thus improving the thermal uniformity. In addition, the fan positions to produce parallel airflows at 0° angle to the capillary array are less space consuming and allow the placements of other accessories to be integrated into the thermocycler system.

Subsequently, the effects of fan volumetric flow-rates, capillary wall thickness, capillary internal diameter as well as the capillary-to-capillary spacing on cooling rate and thermal uniformity are evaluated. Firstly, the fan of volumetric flow-rate of 0.15m³/min is preferred as it provides the fastest rate of cooling.

From the results, it is also noted that by reducing the capillary-to-capillary spacing, the rate of cooling deteriorates while the temperature distribution along x and y-axis are improved. Spacing of 0.5mm or less is preferred to minimize temperature differences along x-axis and y-axis. Capillary wall thickness should be less than 0.1mm to ensure a cooling rate of at least 18°C/s for rapid thermocycling. In addition, even though a large internal capillary diameter will deteriorate the thermal uniformity within the individual capillary (along y-axis), the improvement on the thermal uniformity across the capillary array (along x-axis) is more substantial, and thus an internal diameter of up to 1.8mm is preferred. Even though cooling rate will be compromised due to the larger water volume from a bigger internal diameter, the resultant cooling rate is still faster than the desired cooling rate.

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6.1 Introduction

This chapter deals with the fabrication of the proposed design for the multi-reaction PCR chip and thermocycler system, as well as the evaluation of the IR-mediated thermocycling system and PCR chip for DNA amplification. The fabrication of the PCR chip and the loading procedure for the PCR mix are first explained. Subsequently, initial tests are conducted to establish and optimize the parameters for PID. The PID optimization is crucial for achieving rapid PCR thermal cycling and thermal uniformity, which is vital to successful DNA amplification. Then, experimental tests are carried out to evaluate the performance of the PCR thermocycler and multi-reaction chip in DNA amplification. The PCR chip performs PCR process in the thermocycler system and its results are monitored in real-time. All experimental tests are analyzed and presented in this chapter. Experimental results are also compared with the numerical results in Chapter 4 and 5.

6.2 PCR chip fabrication

The proposed PCR chip design is mainly an array of multi-reaction thin-wall borosilicate glass capillaries. Capillaries offer a low-cost and easily available alternative for PCR mix on-chip containment as opposed to PCR reaction chambers fabricated on chip by other means such as MEMS fabrication processes. Since capillaries can be disposable, the risk of contamination is further reduced. Depending on the diameter of capillary used, a single PCR chip can accommodate more than 30 capillaries. Sample volume ranges from 1 to 12 μl , depending on the type of capillary used. Since the developed PCR thermocycler is suitable for any PCR chip of the standard glass slide format, hence the proposed design for the PCR chip is identical to the size of the glass slide.

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For experimental evaluation of the sample loading and capillary sealing process, a prototype of the PCR chip is fabricated, comprising of an array of 12 thin-wall capillaries (World Precision Instruments Inc., Sarasota, U.S.A.). The capillaries have an internal diameter of 0.75 mm and have to be cut to the desired length of 18 mm. Therefore, each capillary contains approximately 7 μ l of PCR mixture. Even though capillaries in an unopened vial are sterilized, additional preparations are undertaken to ensure minimal risks of contamination, prior to every PCR experiment. The procedures for capillary surface treatment are as follows (see Table 6.1):

Table 6.1: Procedure of capillary surface treatment

S/No	Steps	Purpose
1	Wash and rinse glass capillaries in acetone	Preliminary washing to remove grease
2	Immersed in solution of Conc. Sulphuric acid (H ₂ SO ₄) and Hydrogen Peroxide (H ₂ O ₂)	Immersed for 1 day for very clean capillary
3	Rinse in tap water	Preliminary rinsing to remove residual acid
4	Rinse in deionised (DI) water	Wash away acid
5	Put capillary in oven or hotplate to dry	Drying temperature about 110°C

After surface treatment, the capillaries are ready for the fabrication of the PCR chip. Acrylic is chosen for the base substrate material, as it can easily machined and is commonly available. In addition, acrylic is a good thermal insulator and reduces thermal cross-talk between capillaries. Figure 6.1 presents the dimensions for the base substrate. The base is fabricated by milling. Since the IR-transmitting lens is 50mm long and the diameter of the halogen tungsten light bulb is 10mm, an effective area of 50mm long and 10mm wide will be heated by the light bulb. A window of the same size is cut from the substrate material to allow optical detection of fluorescence emission from the PCR mix by the linear image sensor. The window will also facilitate airflow below the capillaries. Before the positioning of capillaries, the substrate has to be washed thoroughly with ethanol

to remove any grease and other surface contaminants. This is to ensure a hydrophilic surface for capillary action to occur.

From the simulation results in Chapter 5, capillary-to-capillary spacing of 0.5mm or less is preferred. Thus, this spacing depends on the intended user and can be reduced to less than 0.5mm to accommodate more capillaries. In Figure 6.1, the prototype design of PCR chip is shown. This chip is fabricated and used to demonstrate the loading process of the PCR mixture and sealant in section 6.2.1 and 6.2.2.

Figure 6.1: Completed prototype of PCR chip (not to scale)

3M clear double-sided tape is applied to form the walls of the dispensing reservoirs and waste reservoirs on the chip, as shown in Figure 6.1. This tape has been tested and it is able to retain its adhesion when in contact with water-based solution and during elevated temperatures. In addition, a small amount of silicone sealant is applied between the capillaries to form the capillary connectors. These connectors connect the capillaries to form an array and serve as the channel walls for Channels 1 and 2 to prevent leakages. Lastly, a thin glass plate (20mm x 5mm x 0.1mm) is adhered to the top of the capillary using silicone sealant. Caution has

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to be exercised to prevent any sealant from sealing the capillaries prior to sample loading. The PCR chip is completed as seen in Figure 6.1 and 6.2.

Figure 6.2: (a) Photograph of a prototype of the entire PCR chip and (b) zoomed-in view of the capillary array on the same chip.

6.2.1 Loading process of PCR mixture

The following procedure is performed to test for the ease of loading PCR mixture onto the chip. The red liquid represents the PCR mixture and it is composed of water and red dye. The red dye is a powder that consists of high Manganese(Mn) content and produces a red solution when mixed with water. This will allow for easy visualization when loading the mixture in the following illustration. In actual experiment, the PCR mixture is a transparent solution. Figure 6.3 describes the loading procedure of the PCR mixture onto the PCR chip.

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Figure 6.3: Sample loading process for PCR chip

In Figure 6.3, the PCR sample, represented by the red liquid, is initially dispensed into the dispensing reservoir (seen in Figure 6.3a). Capillary action causes the sample to begin to flow into the smaller Channel 1 (Figure 6.3b). Meanwhile, the PCR mixture is dispensed continuously into Dispensing reservoir 1 via pipette and causes the mixture to progress along Channel 1. As the sample reaches the position of each capillary, capillary action will, in turn, cause the sample to flow into the small capillary. Consequently, all capillaries are filled with PCR mixture as the sample flows along Channel 1 (Figure 6.3b to h). In all, the array of 12 capillaries is entirely filled with sample in 2 minutes, as seen Figure 6.3(h).

6.2.2 Sealing of capillaries

During the PCR thermocycling procedure, in the event if the capillaries end are not sealed properly, a loss of sample will be resulted by evaporation, or cross-contamination between adjacent reaction vessels. Hence, a sealant is required to effectively seal the ends of the capillaries while capable of enduring intense thermal cyclic stresses from the thermocycling reaction. Polydimethylsiloxane (PDMS) elastomer is used to fulfill this function. The type of PDMS elastomer used is SYLGARD® 184 silicone elastomer from Dow Corning Corporation [106]. It is designed to protect against moisture, environmental attack, mechanical

and thermal shock as well as vibration especially when optically clear product is required.

6.2.2.1 Preparation of silicone elastomer

The SYLGARD 184 silicone elastomer consists of base and curing agent in separate containers. The two components should be thoroughly mixed using a weight or volume ratio of 10:1 [106]. Both components are mixed with a smooth action that does not introduce excess air. After thoroughly mixing base and curing agent, the mixture is agitated gently to reduce the amount of air introduced. Allowing the mixture to set for 30 minutes before pouring into the PCR chip will aid in the removal of the air introduced during mixing. If air bubbles are still present, vacuum deairing is required. Air entrapped in the mixture can be removed by using a vacuum of 28 to 30 inches Hg.

6.2.2.2 Loading process of sealant

After the PCR mixture is loaded successfully into the capillary array, the capillaries have to be effectively sealed to prevent contamination and evaporation during PCR thermal cycling. The following process is conducted to examine the ease of loading of the silicone elastomer onto the chip. The silicone elastomer is a transparent and viscous solution. Due to its viscosity, it cannot be mixed with coloured dye for easy visualization during the illustration. Figure 6.4 describes the loading procedure of the silicone elastomer onto the PCR chip.

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Figure 6.4: Sealing of the capillary array

In Figure 6.4, we observe the sealing of the capillary array using SYLGARD 184 silicone elastomer. Since the silicone elastomer is a more viscous solution than the water-based red ink, the elastomer will flow at a slower rate. As seen in Figure 6.4(a), the elastomer is pipetted into the Dispensing reservoir 1. The elastomer begins to displace the sample in the dispensing reservoir, as seen in Figure 6.4 (b). In Figure 6.4 (c), the elastomer is pipetted into the Dispensing reservoir 2 after 15 seconds. Similar to Figure 6.3, the elastomer flows along Channel 1 and 2 due to capillary action. In this manner, the sample in Channel 1 is slowly displaced by the elastomer and collected at the waste reservoir 1, where it can be manually

removed with a small slip of absorbant material. The elastomer is unable to flow into the capillaries as the PCR mixture already occupies the capillaries. The sample is completely purged from Channel 1 in about 13 minutes and 33 seconds, as shown in Figure 6.4 (r). Hence, it effectively seals the capillary ends in Channel 1.

On the other hand, the elastomer is able to flow faster in Channel 2, as there is no sample impeding its passage. Channel 2 is filled entirely with elastomer in 4 minutes and 1 second (see Figure 6.4(o)). Elastomer continues to flow and fill up Waste reservoir 2. The silicone elastomer is able to seal the ends of the capillaries at Channel 2.

Throughout the loading procedure, the elastomer is often injected into the Dispensing reservoirs 1 and 2 by adding droplets of elastomer. This could be achieved by accumulating the elastomer at the pipette tip before dispensing it into the reservoir. This is due to the high viscosity of the elastomer, which will frequently trap pockets of air within the pipette tip. By accumulating the elastomer into a droplet, trapped air pockets can escape and thus minimizes the possibility of air bubbles in the elastomer during injection. Each droplet of elastomer is approximately 5 to 10 μL .

During the course of the loading process, the PDMS elastomer is still a liquid mixture. Dow Corning silicone elastomers may be either room temperature (25°C) or heat cured. They may also be heat accelerated for faster cure. The SYLGARD 184 silicone elastomer requires about 4 hours at 65°C to be fully cured [106]. However, for it to be cured sufficiently for handling, the working time at 65°C is about 20 minutes. In this case, upon the sealing of capillary ends with PDMS elastomer, the PCR chip is then transferred to the PCR thermocycler to be incubated at 65°C for 20 minutes. This enables the PDMS elastomer to be sufficiently solidified to effectively encapsulate the capillary ends to prevent sample evaporation and displacement. The incubation temperature is insufficient

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to initiate the denaturation of the dsDNA. After this, PCR reaction begins with an initial hot-start stage of 95°C for 2 minutes and lastly, PCR thermocycling for 30-40 cycles. The initial hot start ensures that all DNA strands have denatured to single stranded DNA and activates the enzyme.

In the capillary sealing process of Figure 6.4, we can observe that the both Channels 1 and 2 are loaded with silicone elastomer in a small time difference of only 15 seconds apart (see Figure 6.4a and c). Hence, the loading of silicone elastomer is almost performed simultaneously. The following are alternative procedures for the loading of silicone elastomer that have also been tested successfully:

1. Channel 2 is loaded and completely sealed first. Thereafter, Channel 1 is loaded with elastomer to purge the sample and seal the capillaries.
2. Channel 1 is loaded and completely sealed first, followed by the loading and sealing of Channel 2.
3. Before the loading of either Channel 1 or 2, the remaining PCR sample in Channel 1 is removed by an absorbant material, suction, air pumping or evaporation. Hence, Channel 1 is initially devoid of the PCR sample before the sealing process of both Channel 1 and 2 begins.

The above-mentioned alternative processes are several sub-options that can be used for the loading of silicone elastomer.

6.3 PCR integrated system

The PCR thennocycler is an automated and integrated system with multiple functions including thermal cycling control, real-time fluorescence detection, and data processing for real-time data analysis. Heating and cooling are realized by alternating infrared (IR) radiation heat transfer and forced convective cooling. The thermocycler system consists of two modules, which are the thermocycling and fluoremetric module as well as the temperature control and data acquisition module. The entire system weighs less than 10 kg and is several times more

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compact as compared to many commercial systems. In this section, the two modules are described in greater detail.

6.3.1 PCR thermocycling and fluorometric module

This module comprises of the heating and cooling elements for PCR rapid thermocycling and the fluorescence detection/imaging system. The photographs of this module are shown in Figure 6.5.

(a) front panel closed

(b) front panel removed

Figure 6.5: Photographs of the thermocycling and fluorometric module when (a) front panel is closed and (b) front panel removed.

The arrows in red identify the components that can be seen in the photograph of Figure 6.5. The view of other components such as excitation wavelength filter, IR-transmitting lens holding assembly, cooling miniature fans and the halogen tungsten light bulb (heat source) are obstructed and are not seen in the photograph.

Figure 6.6: Photographs of plan view of the interior of the thermocycling and fluoremetric module

As seen in Figure 6.6, a long slit (2mm wide and 70mm long) is machined into the PCR chip holding platform and serve as the detection window for fluorescence detection by the linear image sensor. The sensor is located beneath the platform and detects the fluorescence emission from the capillaries through the slit. A narrow slit also helps to ensure that the main bulk of light detected by the linear sensor are from the capillary array and interference from unwanted light sources are avoided. The detection window is situated at a distance of 3mm below the capillaries, which aids to ensure maximum intensity of the fluorescence to pass through the window. In addition, In Figure 6.6, we can also see the placement of the four miniature fans for achieving rapid cooling. The PCR chip seen in this photograph is an early prototype that is fabricated with a bigger spacing of 0.8mm. The detailed schematic diagrams of this module are presented in the following Figures 6.7 and 6.8.

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Figure 6.8: End view of PCR thermocycler design

6.3.1.1 Description of components for the PCR thermocycling and fluorometric module

This module provides the thermocycler with the capabilities to perform rapid thermocycling as well as real-time fluorescence detection. The following are brief descriptions of the components integrated in this unit.

IR radiation source

OSRAM's HALOLINE® Tungsten-halogen lamps (see Figure 6.9) are used as the IR radiation source. It is a cylindrical halogen 500W light bulb that can radiate thermal infrared (IR) energy to the PCR chip. It has a long life span of 2000 hours and has a colour temperature of approximately 3000K [84]. It is dimmable and can be switched ON/OFF almost instantaneously. The radiation source will be switched on during heating process. Conversely, the bulb will be switched off during cooling. Its long cylindrical design will help to ensure a better thermal uniformity along the length of the capillary array.

Figure 6.9: HALOLINE® Tungsten-halogen lamps [84]

Cylindrical Lens

This is a cylindrical lens to image the emitted fluorescence from the PCR product as an elongated line onto the linear image sensor [41]. The lens material is zinc selenide to provide extreme uniformity and homogeneity [66]. This material also allows visible transmission, which simplifies the optical system alignment. The distances between the fluorescence

emission and image sensor have to be calibrated to be able to detect uniform and maximum fluorescence intensity.

IR-transmitting filter

This filter will eliminate wavelengths that could interfere with the PCR reaction, while allowing 92% [83] of 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.

Excitation source

It is also an OSRAM HALOLINE® Tungsten-halogen lamp. However, this cylindrical light bulb has a lower power rating of 150W. This helps to reduce system overheating and power consumption. Its primary purpose is to excite the SBYR Green fluorescent dye intercalated in the amplified DNA strands in order to produce a strong fluorescence signal. Its long cylindrical design will enhance illumination uniformity on the PCR chip. An excitation filter will be used to transmit light of the excitation wavelengths onto the capillary array.

Excitation wavelength filter

SBYR Green dye is used in the experimentations for the detection of successful amplification of PCR products. The excitation wavelength for SBYR Green dye is about 492nm. Therefore, a narrow band-pass interference filter from Edmund Industrial Optics [86] of 488nm is used. The use of the filter also allows the flexibility in the usage of other commercially available PCR fluorescent dyes, such as FAM, Tet, Tamra and ROX. Their excitation wavelengths are 470nm, 510nm, 540, and 547nm respectively [62]. The filters are interchangeable to suit the different excitation needs.

Detection wavelength filter

The detection wavelength for fluorescence emission due to SBYR Green dye is 521nm. In this case, a narrow band-pass interference filter from Edmund Industrial Optics [86] of 520nm is used. Even though the SBYR Green dye emits fluorescence wavelength of about 521nm, there are interferences from other light sources such as light of the excitation wavelength. Hence, the filter will remove light of unwanted wavelengths and greatly improves the specificity of the detection of the intensity of fluorescence emission from the PCR products. As mentioned before, filters allow the flexibility in the usage of other commercially available PCR fluorescent dyes, such as FAM, Tet, Tamra and ROX. The filters are interchangeable to suit the different detection needs.

Linear image sensor

This device detects the fluorescence emission from successful PCR products in real-time. In the thermocycling and fluoremetric module design, NMOS S3901-1024Q linear image sensor is used [95]. The NMOS linear image sensor (see Figure 6.10) is a self-scanning photodiode array designed specifically as detectors for multi-channel spectroscopy. The scanning circuit is made up of N-channel MOS transistors, operates at low power consumption and is easy to handle. Each photodiode has a large active area, high UV sensitivity yet low noise, delivering a high S/N even at low light levels. The current output type NMOS linear image sensors also feature excellent output linearity and wide dynamic range. The S3901-1024Q uses photodiodes 2.5mm in height and arrayed at a spacing of 25 μ m. The photodiode array has a quantity of 1024 pixels. The main limiting factor to the size of this module is the size of the fluorescence detection unit. In this case, the linear image sensor, when attached to the driver/amplifier circuit, is sufficiently small in size (approximately 89mm

long by 66mm wide by 32mm height), thus enabling the module to be compact in design.

Figure 6.10: NMOS S3901-1024Q linear image sensor [95]

MOS Driver/Amplifier circuit

The C7615 MOS Driver/Amplifier circuit is a low-noise driver/amplifier circuit (see Figure 6.11) designed specifically for use with the NMOS S3901-1024Q linear image sensor. The C7615 driver/amplifier circuit includes a pulse generator to provide a start pulse and two-phase clock pulse to drive a MOS linear image sensor and a signal processing circuit to read out the image sensor video signal in current-integration mode [96]. It only requires a $\pm 5V$ and $\pm 15V$ power supply and digital signal inputs to provide a Start pulse and CLK pulse. Other advantages include low noise output and compact size. Its dimensions are 89mm long x 66mm wide x 2mm thick.

Figure 6.11: Plan view of MOS Driver/Amplifier circuit [96]

Miniature cooling fan

This is a DC fan, as seen Figure 6.12, with electronically commutated external rotor motor, with fully integrated commutation electronics. With electronic protection against reverse polarity, the fan only operates when the polarity is correct. In addition, it is impedance-protected against blocking and overloading. The fan is made of fiberglass reinforced plastic, for enhanced durability and lightweight. Its mass is approximately 17 g [89].

Figure 6.12: Miniature cooling fans [89]

Outer casing

Ambient light could interfere with the detection by the linear image sensor. Hence, the outer casing is an enclosure to prevent interference from ambient light. The material of the casing is black painted aluminum sheet metal. The black-painted surroundings also help to enhance the intensity of the emitted fluorescence, thus improving the detection sensitivity of the linear image sensor.

6.3.2 Temperature control and data acquisition module

This module comprises of the PID temperature controller, the circuitry of solid-state (SS) relays and DC power supply, and lastly, the data acquisition and processing unit (see Figure 6.13).

Figure 6.13: Photographs of the temperature control and data acquisition module

6.3.2.1 Description of components of PCR temperature control and data acquisition module

Temperature controller

The Watlow Series 982 vertical 1/8 DIN-ramping temperature controller shown in Figure 6.14 is selected as part of the temperature control and data acquisition module. The Series 982 is an easy-to-use ramping controller and is designed to meet the needs of most ramping applications [97]. Ramping operations include four files with six steps in each file, whereas programming options include ramp-rate or time-based profiles, guaranteed soak deviation, program looping and program status selection after power outage. Its primary analog input accepts up to 11 different thermocouple types, RTD or scalable process inputs. With a maximum of two event inputs, the SERIES 982 is capable of remote program start or hold functions and allows the operator to program a wait-for event. The

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SERIES 982 comes packaged with a NEMA 4X front panel to withstand harsh environments, four inch case depth and touch-safe wiring terminal.

Figure 6.14: Watlow Series 982 vertical 1/8 DIN-ramping temperature controller [97]

Data acquisition/processing unit

The C8799 from Hamamatsu (Figure 6.15) is a data processing unit that converts video signals from the NMOS image sensor driver circuit into digital signals and transfers them to a PC. It has USB interface which allows easy connection to a PC for high-speed data communications at 12 Mbps. Other features include a built-in 12-bit A/D converter, an adjustable data rate (5 MHz max), internal memory of 1 mega-word [99].

Figure 6.15: C8799 data processing unit [99]

Power supply (15Vdc)

This is an AC to DC power supply for the miniature cooling fans, the C7615 MOS Driver/Amplifier circuit and the C8799 data processing unit. It requires a universal input of 85-264 VAC and 50/60Hz. It is a triple

output model, which are ± 5 and 12 Vdc, and has short circuit and over-voltage protection. It also comes in a compact metal case with screw terminal block [100].

Solid-state relay (SSR)

The solid-state relay (SSR) has no moving parts. It is a semiconductor switch, which conducts load current to the device when the SSR is energized by the control output. The great advantage of SSR is its capability to achieve very short cycle time of between $\frac{1}{2}$ to 1 second [101]. This will allow the PID controller, such as the Watlow ramping controller, to react rapidly to temperature fluctuations. In addition, without moving parts, the SSR is less likely to experience mechanical breakdown and generate less electrical noise during operations.

6.3.3 PCR thermocycler process flowchart

Figure 6.18 describes the system flowchart of the 2 modular thermocycler design.

Figure 6.16: Flowchart of PCR system processes

6.3.4 Size comparison of developed PCR thermocycler with other commercial models

A compact PCR thermocycler design allows the possibility of being portable. Hence in this section, the developed PCR thermocycler is compared with two popular commercial thermocycler models that are capable of real-time fluorescence detection during PCR thermocycling.

Figure 6.17: Size comparison with the LightCycler developed by Roche Applied Science (left) and the Rotor-gene 3000 developed by Corbett Research (right)

From Figure 6.17, the developed thermocycler is significantly more compact and portable as compared to the two models shown.

6.4 PID parameters for PCR thermocycling

As mentioned before, PID control is crucial for attaining rapid thermocycling with minimal temperature fluctuations and overshoots/undershoots. Thus, it is a precise form of temperature control to stabilize workload temperature by adjusting the proportional, integral and derivative parameters. It is also essential to fine-tune the PID parameters in order to achieve a stable and fast thermocycling temperature profiles. Prior to PID parameters optimization, the temperature controllers have to be calibrated first to ensue consistent and accurate temperature measurement.

6.4.1 Thermocouple calibration

The following is the procedure for the calibration of the temperature controller [101].

Equipment Required

- Type K thermocouple calibrator set at 0°C
- Precision millivolt source, 0 - 50mV minimum range, 0.01mV resolution

Setup and Calibration

1. Connect the AC voltage L1, L2 and ground to the proper terminals on the Series 982 Watlow temperature controller.
2. **For Input #1 calibration:** Connect the millivolt source to terminal #9 (+) and terminal #10 (-) on the 982 terminal strip. Set the Input 1 DIP switches to: 1 OFF 2 ON 3 ON
For Input #2 calibration: Connect the millivolt source to terminal #19 (+) and terminal #20 (-) on the 982 terminal strip. Set the Input 2 DIP switches to: 1 OFF 2 ON 3 ON
3. Apply power to the unit and let it warm up for 15 minutes. After warm-up, put the unit in the CAL menu.
4. At the A 50 prompt, or the B 50 prompt, enter 50.00mV from the millivolt source to the Series 982. Allow at least 10 seconds to stabilize. Press the Up key to change the upper display to YES. Press the Mode key.
5. At the A 00 prompt, or the B 00 prompt, enter 0.00mV from the millivolt source to the Series 982. Allow at least 10 seconds to stabilize. Press the Up key to change the upper display to YES. Press the Mode key.
6. At the tc prompt, disconnect the millivolt source, and connect the reference compensator to terminal #9 (+) and terminal #10 (-) on the 982 terminal strip. Allow 10 seconds for the control to stabilize. Press the Up key to change the upper display to YES. To conclude the T/C calibration, press the Display key.

Thereafter, the temperature controllers are calibrated successfully.

6.4.2 PID optimization

After calibration of the temperature controller, PID parameters can be optimized. In Figure 6.18, we can observe two typical PCR thermal cycling profiles with little (left) and excessive (right) PID control on the system. The incubation time for denaturation step is set to be 10 seconds, while annealing and extension step are set at 15 seconds. The thermocouple is immersed in the centermost capillary containing the PCR mix, and a temperature profile based on the measurement of the sample's temperature can be obtained. Subsequently, PCR thermocycling takes place by subjecting the capillary array to a series of radiative heating and convective cooling, controlled by the temperature controller.

Figure 6.18: Thermocycling profile with little PID control optimization (left) and with excessive PID control (right)

Figure 6.18 (left) shows the thermal cycling profile before optimizing the PID parameters. Large overshoots and temperature fluctuations (about $\pm 5^{\circ}\text{C}$) around set temperatures can be observed. Each complete cycle (denaturation-annealing-extension) takes about 65 seconds. Figure 6.18 (right) shows a smooth temperature profile resulted by applying excessive PID control. This set of PID parameters results in a longer time to reach the set temperature, as each cycle takes approximately 90 seconds to complete. However, this is also the best temperature profile achievable and is very stable. Fluctuations are minimal at less than 1°C about the set temperature.

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Therefore, it is necessary to find an optimal temperature profile for the PCR thermal cycling that is capable of a rapid and sufficiently smooth and stable profile. The PID parameters are adjusted and fine-tuned until an optimized temperature profile with small overshoots/undershoots of short durations and little fluctuations is attained, as seen in Figure 6.19.

Figure 6.19: Thermocycling profile after PID optimization

Figure 6.19 shows a temperature profile with small overshoots/undershoots of short duration ($=1$ sec) and fluctuations. The maximum overshoots/undershoots are about $\pm 2^{\circ}\text{C}$ and fluctuations of less than $\pm 1^{\circ}\text{C}$ about the set temperature. A complete cycle takes about 70 seconds. As a result, a PCR experiment of 30 cycles can be completed in about 35 minutes.

A comparison is also made between the temperature of the capillary exterior surface and the temperature of the PCR mixture. In this case, the capillary used is of 1 mm internal diameter, which is sufficient for the thermocouple to be immersed in. The results are presented in Figure 6.20.

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Figure 6.20: Temperature profile measured at the capillary exterior surface (left) and within the PCR mixture (right).

Figure 6.21: Comparison between temperature profile of capillary interior and exterior on a single plot

Figure 6.21 is a comparison of the plots in Figure 6.20 and shows the temperature profiles measured at the capillary exterior surface and within a capillary on a single plot. As we can observe, the profile measured within the capillary is a smoother profile with less fluctuations and overshoots/undershoots, compared to the profile measured at the capillary surface. This is likely due to the thin-wall capillary (wall thickness of less than 0.2mm depending of the type of capillary), thermal mass of capillaries are very small and allows the temperature ramping of the liquid sample to be close to that at the capillary exterior. From our measurements, the delay in temperature of liquid sample inside a PCR well

compared to the temperature measured at the capillary exterior is minimal. The overshoots/undershoots and the temperature fluctuation in PCR sample are reduced to less than 1°C and 0.5°C , respectively. As seen in the profile obtained at the exterior surface, an initial overshoot or undershoot is required to speed up the temperature change in sample contained in reaction wells. However, it is well controlled to keep it within a proper limit and duration; otherwise negative effects to PCR amplification might be resulted.

Hence, from the comparison in Figure 6.21, PCR thermocycling can be based on the temperature measurement of the capillary exterior surface to obtain a PCR sample temperature with less fluctuations and reduced overshoots/undershoots. This will also allow capillaries of smaller diameter, that the thermocouple is unable to insert in, to be used.

6.5 Experimental test of temperature uniformity within the capillary array

Temperature uniformity on the PCR chip is an important factor in achieving consistent DNA amplification for multiple, consecutive reactions. A dual thermocouple setup was initially used to determine the temperatures at various positions along the length of the capillary array. In this setup, a thermocouple is situated at a designated position on the PCR chip and another thermocouple is fixed at the center capillary. A total of 5 tests are carried out for 5 different positions. Both thermocouple are calibrated and presented consistent temperature measurements. For this test, excessive PID control is used. The results are presented below for comparison.

Figure 6.22: Temperature profile at (a) center capillary of PCR chip and (a) 5mm from center capillary

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Figure 6.23: Temperature profile at (a) center capillary of PCR chip and (a) 10mm from center capillary

Figure 6.24: Temperature profile at (a) center capillary of PCR chip and (a) 15mm from center capillary

Figure 6.25: Temperature profile at (a) center capillary of PCR chip and (a) 20mm from center capillary

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Figure 6.26: Temperature profile at (a) center capillary of PCR chip and (a) 25mm from center capillary

Figure 6.27: Comparison between temperature profiles at different distances from the center in a single plot.

Figure 6.27 is a superposition of Figures 6.24 to 6.28 onto a single plot. In Figure 6.27, we see that temperature profile at 5mm and 10mm from the center shows little temperature difference and time delay. However, we can begin to observe an increase in temperature difference in profiles at 15mm from the center when compared with the profile obtained at the center (0mm). The delay in temperature change is about 1-2 second. The overshoots/undershoots and the temperature fluctuation at 10mm from the center are at approximately 1-2°C and 0.5-1°C, respectively. We can also observe that the temperature difference is more prevalent and the response of temperature change becomes slower when the

distance from center increases. At 20mm from the center, the overshoots/undershoots and the temperature fluctuations are 1-2.5°C and 0.5 to 1.5°C respectively. The temperature profile at 20mm is approximately 1 to 3 seconds slower than the center. The overshoots/undershoots of profiles up to 20mm distance are of very short duration of less than 1 second. At the 25mm distance, the delay in temperature change can be up to 10 seconds. Overshoots/undershoots (mainly undershoots) and the temperature fluctuation at 25mm from the center are at approximately 5°C (mostly undershoots) and 2-4°C, respectively.

Therefore, we see that the problem of temperature overshoots/undershoots and temperature fluctuations are significantly worsened after the distance of 20mm. Response time to temperature change also becomes slower. Based on these experimental findings, the dotted rectangular box of 40mm length in Figure 6.28 indicates the effective area for the placement of capillary array to have good temperature uniformity. This area is smaller than the area predicted by the numerical results in Chapter 4 and 5. Thus, the final PCR chip design is shown in Figure 6.28. For capillaries of 1mm internal diameter at capillary-to-capillary spacing of 0.1mm, the maximum number of capillaries that can be accommodated in the PCR chip is 32.

6.6 Comparison between numerical analysis results and experimental results

In this section, the results from numerical solutions in Chapter 4 and 5 are compared with results from experimental validation. Experimental data presented in Figures 6.29 and 6.30 are obtained from real-time temperature monitoring by thermocouple during PCR thermal cycling. A type K thermocouple (Watlow Electric Manufacturing Co., U.S.A.) of about 0.5 mm in diameter is immersed into the centermost capillary of capillary array. Capillaries (World Precision Instruments Inc, U.S.A.) used have internal and external diameters of 0.75mm and 1mm respectively. PID optimized thermal cycling is performed with the thermocouple measured the temperature within the centermost capillary.

Dimensions of capillary array in the numerical simulations are exactly the same as the actual array used in experiments. Both experimental and numerical measurements are obtained at identical locations of the capillary array and compared in Figures 6.29 and 6.30.

Figure 6.29: Comparison of experimental data and numerical solutions on rate of heating

Figure 6.29 is a comparison between experimental results and numerical solutions on the rate of heating during the transition of the extension process (70°C) to the denaturation process (55°C). From the experimental data, the temperature increases at a slower rate due to the control of the PID. Hence, we can see that as the temperature approaches 90°C , the increase in temperature becomes slower, as the PID controls by switching off the heat source frequently as well as reducing the power supplied to it. However, by looking at the initial rate of increase (represented by the dotted line), the experimental data can achieve 95°C in about 3.7 seconds, as compared to 2.6 seconds that is predicted by numerical analysis. The time difference is about 1.1 seconds. This could be due to a slight over-estimation of the power supply of the heat source during the numerical solutions, thus resulting in a higher ramping rate.

Figure 6.30: Comparison of experimental data and numerical solutions on rate of cooling

In Figure 6.30, we have a comparison of experimental data and numerical solutions on the rate of cooling during the transition of denaturation process (95°C) to annealing process (55°C). Similarly, the experimental data in Figure 6.30 shows a slower decrease in temperature. This is also because of the control of the PID. In this case, as the temperature approaches 58°C , the PID controls by switching off the fans more frequently. However, by looking at the initial rate of

decline (represented by the dotted line), the experimental data can achieve 55°C in about 9 seconds, as compared to 3 seconds that is predicted by the numerical analysis. The time difference is significant at about 6 seconds. This could be due to the simplified modeling of the interior structure of the thermocycling chamber. Structures, such as the IR lens holding assembly and PCR chip holding platform are modeled as simple rectangular 3-D blocks. In addition, the PCR chip is modeled simply as the capillary array, instead of the entire chip structure. The simplified modeling is to reduce the possibilities of errors during mesh generation and solution, as well as for the ease in modeling. However, these constitutes to the inaccuracies in the solutions.

Even though there are discrepancies in the results of experimental evaluations and numerical solutions, the numerical results allow an early insight of the performance of the proposed designs before carrying out any time-consuming and costly fabrication process and procurement of equipments and materials. These results enable time-savings and provide an advantage in avoiding any possible pitfalls in the thermocycler and chip design. In this case, the numerical results show the possibility of the thermocycler and chip design in achieving rapid thermocycling and thermal uniformity.

6.7 Initial evaluation of the IR-mediated thermocycling system and multi-reaction PCR chip for PCR-based amplification of DNA utilizing Human DNA

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

6.7.1 PCR mixture preparation

The PCR mixture is prepared for 6 capillaries at capillary-to-capillary spacing of 0.5mm. Sample volume per capillary is 7 μ l.

Table 6.2: Recipe for PCR mixture

Master Mixture	1X Reaction	x+2 = 10 Samples	Final Concentration
Forward Primer (10 μ M)	0.35 μ l	3.5 μ l	0.5 μ M
Reverse Primer (10 μ M)	0.35 μ l	3.5 μ l	0.5 μ M
dNTPs (10 mM)	0.14 μ l	1.4 μ l	0.2 mM
BSA (10 mg/ml)	0.7 μ l	7 μ l	
Buffer (10X)	0.7 μ l	7 μ l	1X
MgCl ₂ (50 mM)	0.28 μ l	2.8 μ l	2 mM
SYBR Green (100X)	0.28 μ l	2.8 μ l	4X
MQ Water	2.8 μ l	28 μ l	
Pt <i>Taq</i> DNA polymerase (5U/ μ l)	0.7 μ l	7 μ l	0.5 U
Volume	6.3 μ l	63 μ l	
DNA Template [3.7 ng/ μ l]	0.7 μ l	7 μ l	0.37 ng/ μ l
Total Volume	7 μl	70 μl	

The second column in Table 6.2 describes the composition of the PCR mix that is sufficient for sample volume of one capillary. The third column shows that total PCR mix volume for 8 capillaries to be prepared. The extra capillary volumes are prepared to accommodate the wastage during sample loading. The DNA copy number in the mix is 1000.

6.7.1.1 Descriptions of each component in the PCR mixPrimer-pairs for PCR

The PCR primers pairs used in this experiment has a length of 25 bases and are designed to flank the region of amplification. The primers are designed to avoid sequences, which would produce internal secondary structures. Ideally, both primers should anneal at the same temperature.

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The sequence of the β -acting forward primer (Promega Cat. #G5740, Research Instruments Pte. Ltd., Singapore) is 5'-TCACCCACACTGTGCCCATCTACGA-3'.

The sequence of the reverse primer includes regions at the 5'-ends, which can be useful for downstream applications. Sequence of β -acting reverse primer is (Promega Cat. #G5740, Research Instruments Pte. Ltd., Singapore) 5'-CAGCGGAACCGCTCATTGCCAATGG-3'. Regardless of primer choice, the final concentration of the primer in the reaction mix is at a final concentration of 0.5mM in a 7 μ l reaction.

dNTPs – Promega Nucleotide Mix

PCR Nucleotide Mix (Promega Cat. # C1141, Research Instruments Pte. Ltd., Singapore) is a premixed solution containing the sodium salts of dATP, dCTP, dGTP and dTTP, each at a concentration of 10mM in water. This solution is ready to use and is optimized for standard polymerase chain reactions and specialty approaches including hot-start and reverse transcription PCR (a) (RT-PCR). The final concentration of the dNTPs in the reaction mix is at a final concentration of 0.2mM in a 7 μ l reaction.

Bovine Serum Albumin (BSA), Acetylated

Acetylated BSA (Promega Cat. #R396A, Research Instruments Pte. Ltd., Singapore) can be used as an enzyme stabilizer or as a carrier protein. It is dialyzed extensively with deionized water to remove impurities.

Taq DNA Polymerase PCR Buffer

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

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

Magnesium Chloride (MgCl₂)

Magnesium concentration is a crucial factor affecting the performance of *Taq* DNA polymerase. Reaction components, including template DNA, chelating agents present in the sample (e.g., EDTA or citrate), dNTPs and proteins, can affect the amount of free magnesium. In the absence of adequate free magnesium, *Taq* DNA polymerase is inactive. Conversely, excess free magnesium reduces enzyme fidelity and may increase the level of nonspecific amplification. For these reasons, it is important to empirically determine the optimal MgCl₂ concentration for each reaction. Thus, the Mg²⁺ concentration is optimized for each reaction. In this case, the final concentration of the MgCl₂ (Invitrogen Cat. No. 10966-018, Invitrogen Singapore Pte. Ltd., Singapore) in the reaction mix is at a final concentration of 0.2mM in a 7µl reaction.

SYBR Green

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

MQ Water

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

Platinum® *Taq* DNA Polymerase

Platinum® *Taq* DNA Polymerase (Invitrogen Cat. No. 10966-018, Invitrogen Singapore Pte. Ltd., Singapore) is recombinant *Taq* DNA

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polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the denaturation step in PCR cycling at 94°C, thereby providing an automatic hot start for *Taq* DNA polymerase in PCR. Hot starts in PCR provide increased sensitivity, specificity, and yield, while allowing assembly of reactions at room temperature. The use of this antibody helps reduce PCR optimization requirements, reaction set-up time and effort, handling of reaction components, and contamination risk, thereby improving PCR results. Platinum® *Taq* DNA Polymerase is supplied at the same 5 unit per µl concentration as *Taq* DNA Polymerase. No modifications to PCR reactions or protocols are necessary. This enzyme formulation can also be used in larger volume cocktail mixes without difficulty.

DNA Template

The human DNA template used in this experiment is Homo sapiens BAC clone CTB-161C1 from 7, complete sequence. The following sequence is obtained from the BLAST database in National Center for Biotechnology Information (NCBI) [107].

tcaccaca	ctgtgccat	ctacgagggg	tatgccctcc	cccatgccat
cctgcgtctg	gacctggctg	gccgggacct	gactgactac	ctcatgaaga
tcctaccga	gcgcggctac	agcttcacca	ccacggccga	gcgggaaatc
gtgcgtgaca	ttaaggagaa	gctgtgctac	gtcgccttg	acttcgagca
agagatggcc	acggctgctt	ccagctctc	cctggagaag	agctacgagc
tgctgacgg	ccaggtcatc	accattggca	atgagcggtt	ccgctg

6.7.2 PCR Experimental results and discussions

The PCR mix can be prepared based on the recipe presented in section 6.7.1. For the evaluation, a PCR chip of 6 capillaries is fabricated as seen in Figure 6.31.

Figure 6.31 : Plan view of PCR chip of 6 capillaries

Capillaries 1, 4, 6 will be loaded with the PCR mix of positive control. Capillaries 1 and 6 are placed at both sides of the effective area for temperature uniformity to test if DNA amplification can be performed at these locations. The PCR mix described in Table 6.2 is indicated as positive control. Capillaries 2, 3 and 5 are filled with PCR mix of negative control. That means certain ingredient of the PCR mix is removed. In this case, Capillary 2 is filled with the PCR mix without DNA template. Capillary 3 is filled with the PCR mix without primer pair. Capillary 5 is filled with the PCR mix without Platinum *Taq* DNA polymerase. To achieve these, the capillaries are preloaded with their respective PCR mix before being fixed onto the PCR chip substrate. When silicone elastomer flows along Channel 1, the preloaded mix in the capillaries prevents the elastomer to enter the capillary array. On the other end of the capillary, strong hydrogen-bonding interactions with the capillary interior surface prevents the PCR mix to flow into Channel 2. Hence, capillaries are loaded and sealed successfully.

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Upon successful loading of the PCR mixture into the PCR chip, PCR experiment can be carried out. The following Table 6.3 reflects the parameters of the PCR experiment that is carried out.

Table 6.3: PCR experimental parameters

S/No	Stage	Temperature (°C)	Time
1	Curing	65	20 minutes
2	Hot Start	95	2 minutes
3	Denaturation	95	10 seconds
4	Annealing	55	15 seconds
5	Extension	72	15 seconds
6	Final Extension	72	10 minutes
	Number of Cycles	40	

The initial curing stage is to cure the PDMS silicone elastomer sufficiently to be able to seal the capillaries effectively. Thereafter, hot-start PCR begins. Results from real-time fluorescence detection during the PCR experiment are collected and presented below in Figure 6.32.

Plot of fluorescence intensity (FI) vs cycle number

Figure 6.32: Fluoremetric plot of fluorescence intensity (FI) vs cycle number

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As seen in Figure 6.32, fluorescence increase is detected in the positive control PCR mix in capillary 1, 4 and 6. The increase in fluorescence begins at Cycle 17. A higher increase in fluorescence intensity can be seen beyond Cycle 20 and peaks near Cycle 34. Capillary 2, 3 and 5 exhibits some fluorescence. This is because the primers and/or DNA templates present in the negative control PCR mix are strands of DNA, in which SYBR Green dye binds to these DNA strands and fluoresces. Since there is no DNA amplification seen in the negative control PCR mix, no fluorescence increase is observed. In addition, this indicates that there is no cross-contamination between adjacent capillaries during the PCR mix loading procedures and the PCR run.

The linear image sensor has 1024 pixels in the active area (see Figure 6.33); each pixel is a micro-sized photodiode.

Figure 6.33: Plan view of image linear sensor

Experimental results in Figure 6.32 are also plotted with respect to pixel number in the image linear sensor, as shown in Figure 6.34.

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Figure 6.34: Fluoremetric plot of fluorescence intensity (FI) vs pixel number

In Figure 6.34, we see three distinct peaks of the three capillaries loaded with PCR mix of positive control, namely capillary 1, 4, and 6. There is no amplification seen from the capillaries of negative control. Results in Figure 6.32 and 6.34 shows successful amplification of the samples with positive control. From this, we can also observe negligible or no cross contamination occurred between adjacent capillaries (for example, between capillaries 2,3,4 and 5).

A similar batch of PCR mix is used in the Rotor-gene developed by Corbett Research. Results from developed thermocycler and Rotor-gene are shown in Figure 6.35. Parameters for the PCR run are similar to those seen in Table 6.3.

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Figure 6.35: Plotted results obtained from developed thermocycler (left) and from Rotor-gene developed by Corbett Research (right)

From Figure 6.35, we can observe that both profiles show an increase in fluorescence intensity begins at cycle 17. The slight fluctuations observed in the profile in Figure 6.35 (left) are likely due to the presence of electrical noise in the driver circuit of the linear image sensor that obscures the clarity of the signal measured. Figure 6.35 indicates that the developed thennocycler has successfully amplified the DNA sequence at the specific targeted region, as both plotted results show an increase in fluorescence at the 17th cycle.

The PCR mix, identical to the recipe used in the above experiment, is also used in the PTC-100 thennocycler, developed by MJ Research Inc. Parameters for the PCR run are similar to those seen in Table 6.3. The results are checked by running a portion of the amplified PCR mixture in agarose gel electrophoresis. A band representing the amplified DNA is visible after ethidium bromide staining, as seen in Figure 6.36. A photograph of the results from the agarose gel taken after electrophoresis is shown below in Figure 6.36.

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Figure 6.36: Conventional amplification products of a negative control with no template DNA (Lane 1), a positive control with 37 ng/ μ l commercial human blood DNA (Lane 2-5). M indicates the 100 bp molecular weight markers.

Hence, from Figure 6.36, bands are visible only in Lane 2 to 5, as they are PCR products with human blood DNA template added. The bands seen at the bottom indicate the primer-dimer formation. Formation of primer-dimer is common and, together with the specific products, is strongly associated with entry of the PCR into the plateau phase [82]. From these results, we see that the recipe of PCR mix is prepared properly and is capable of DNA amplification by PCR.

In addition, several recipes for the PCR mix, each having different DNA copy number, are prepared. PCR tests are carried out with the developed PCR thermocycler with these different PCR mix and their results are plotted in the following Figure 6.37.

Figure 6.37: Comparison of PCR mix of different DNA copy number

Results of PCR reaction carried out on the developed thermocycler are presented in Figure 6.37. PCR mix of lower DNA copy number exhibits a later fluorescence increase. We can see a fluorescence increase from PCR mix of 1000 DNA copy number at the 17th cycle, whereas the PCR mix of 100 DNA copy number see an increase in fluorescence in the 22nd cycle. Lastly, the PCR mix of 50 DNA copy number has a fluorescence increase beginning at the 28th cycle. This is because a PCR mix of low DNA copy number will have a low final concentration of DNA template; hence, longer cycles of amplifications are required to multiply the DNA template to noticeable levels.

Hence, PCR are performed successfully using the developed PCR thermocycler and multi-reaction PCR chip.

Chapter 7 CONCLUSION

7.1 Summary

In the present research work, an inexpensive and disposable multi-reaction PCR chip has been designed to perform thermal processes required by PCR reaction. This PCR chip requires no costly and time-consuming fabrication processes, such as MEMS techniques. Each chip requires about 30 to 60 minutes to be fabricated, depending on the number of capillary required. It is designed with an array of glass borosilicate capillaries, each capable of containing 1 to 12 μ l of PCR mixture. The sample volume is less than the volume required in most conventional PCR devices. Therefore, the cycle times during PCR and the costs for the expensive PCR reagents could be greatly reduced.

Due to the size of the capillaries as the PCR reaction chambers, more capillaries could be accommodated on a PCR chip, thus throughput could be increased significantly. It is an important feature in large scale DNA screening where a lot of information is being processed. Due to the miniaturizing of the structure of the PCR chip, the heating and cooling rates of the chip can be improved and thus rapid thermal cycling could be achieved and temperature uniformity within the sample could be ensured. Both PCR thermocycler and PCR chip design have also been verified and supported by extensive 3-D modeling, followed by FEM and CFD simulations.

A compact PCR thermocycler is designed and fabricated for evaluation by performing several thermocycling experimental tests. This thermocycler design is based on a PCR chip identical to the size of a standard glass slide. Hence, it could be used for other PCR chip design of the same size. This allows greater flexibility and can serve as a testing platform for PCR chip design development, such as the micro-machined thermal reactor developed by Zou et. al. [21], which is also fabricated on a standard glass slide.

Chapter 7: CONCLUSION

The design of the PCR chip has also been described. The fabrication processes of PCR chip are simple and inexpensive. As a result, the cost of fabrication is much reduced. An initial attempt is made to fabricate the PCR chip with few capillaries. The prototypes of the PCR chip and thermocycler are tested experimentally for their feasibility and performance. A PCR process of 40 cycles is conducted with a standard protocol as the PCR mix. The linear image sensor on-board the thermocycler has demonstrated its ability to detect the amplified PCR products successfully. Subsequently, the developed PCR chip and thermocycler are able to perform rapid PCR and real time detection.

7.2 Conclusions

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

- FEM and CFD analysis results developed in the present work could be applied to study thermal characteristics of the PCR chip and thermocycler from radiation heat transfer and convective cooling. The modelling can also serve as a guide for dynamic calibration of the temperature sensor.
- Based on solutions from these simulations, a design for the PCR chip and thermocycler that is capable of attaining rapid thermal cycling and thermal uniformity is proposed.
- The PCR chip utilizes an array of capillaries as multiple reaction vessels.
- The volume of the capillaries would result in cost reductions and faster thermal cycles. Higher throughput could be achieved. Capillaries are low-cost and allow the possibility of a disposable PCR chip.
- Fabrication processes for the PCR chip are established. Prototypes of PCR chip and thermocycler are fabricated.
- Successful PCR products detected. PCR mixture of the same batch is used to run in other commercial thermocyclers consecutively with the developed thermocycler. Their results are compared and the cycle of amplification coincides. Hence, the PCR chip and thermocycler have performed PCR successfully.
- Rapid PCR thermocycling and real-time detection is achieved.

Chapter 7: CONCLUSION

Table 7.1 below addresses all issues of PCR chip development mentioned in Chapter 3.

Table 7.1: Capabilities of proposed PCR system

	Design requirements for PCR system	Capability of proposed PCR system
1	Precise temperature control	At edge of capillary array, overshoots/undershoots are at approximately 1.5 to 2°C at very short durations of less than 1 second. At bulk of capillary array, overshoots/undershoots are at approximately 1 to 1.5°C at very short durations of less than 1 second. At capillary exterior surface, overshoots/undershoots are at approximately 1 to 2°C at very short durations of less than 1 second. At capillary interior, overshoots/undershoots are at approximately 1 °C at very short durations of less than 1 second.
2	Thermal mass of the PCR system	Small thermal mass. Samples are contained in glass capillaries of 0.75mm inner diameter and 18mm long.
3	Leakages	Little or no leakages as capillaries are sealed at both ends by SYLGARD 184 silicone elastomer.
4	Bubbles formation	Caution must be exercised during sample loading to prevent air bubbles to be trapped within the sample flow.
5	Uniformity of sample temperature	Temperature fluctuations within sample at edge of capillary array are at approximately 0.5 to 1°C. Temperature fluctuations within sample at bulk of capillary array are at approximately 0.5°C.
6	Cycle times	A complete cycle takes about 70 seconds. 30 PCR cycles can be completed in about 35 minutes.
7	Amplification of desired molecules	Fluorescence intensity increases with increasing thermal cycles. Successful amplification of desired molecules detected
8	Transition time between temperatures	Transition times ranging from 5.2 to 9 seconds.
9	Costs	Low cost capillaries. Disposable PCR chip.
10	Throughput	High throughput. As many as 30 capillaries can be accommodated depending on the diameter of capillaries used.

7.3 Recommendations and future work

Since PCR is a rapid thermocycling process, it also becomes a likely cause of failure for the material used by the PCR thermocycler, particularly, the IR transmitting filter. The filter is directly exposed to the halogen light bulb in close proximity, and has to withstand large amounts of cyclic stresses due to the fluctuating temperatures of vastly different temperatures during PCR. In the course of many experimental tests conducted on the thermocycler, the IR filter

Chapter 7: CONCLUSION

had experienced a fracture and had been replaced. To remedy, the filter is positioned further from the heat source. However, due to compact design, the filter is positioned at the furthest allowable distance from the heat source. Inevitably, this will only delay another occurrence of fracture and thus, a solution to prevent further occurrence is required.

A possible solution is to use a halogen light bulb of a smaller power rating. Power ratings of 150W and 300W light bulb are readily available and could be used. This, coupled with the positioning of the filter, will help to significantly reduce the cyclic stresses experienced by the filter. In addition, a filter holding assembly that can allow easy removal and replacement of the IR transmitting filter is advised. Hence, the filter holding assembly should be remodified to allow this function.

Future work for this project will also be primarily on the quantitating of DNA amplicons during the fluorescence detection stage and the fine-tuning of the PCR system to achieve greater stability and reliability.

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Appendix A – CALCULATION OF FOCUSING DISTANCE OF LENS

APPENDIX A – CALCULATION OF FOCUSING DISTANCE OF LENS

This appendix presents the calculations for the focusing distances used in the PCR system set-up. The following figure depicts the distances between the capillary of the PCR chip and the linear image sensor.

Equations

The relationship between the focal length and the focusing distances is given by

$$\frac{1}{x} + \frac{1}{d - x} = \frac{1}{f} \quad (a)$$

$$\text{Magnification factor, } M = \frac{d - x}{x} \quad (b)$$

Calculations

Magnification would result in a loss in the focusing intensity of the fluorescence from the capillaries. Hence, to ensure no magnification, $M = 1$.

i.e. $d = 2x$

Since focal length, $f = 25$ mm, thus $x = 25$ mm.

To satisfy equations (a) and (b), $x = 50$ mm and $d = 100$ mm

Appendix B – CONTROL SOFTWARE ARCHITECTURE

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Legend

Appendix C – PROPERTIES TABLES

APPENDIX C – PROPERTIES TABLES

TABLE A.4 Thermophysical Properties
of Gases at Atmospheric Pressure^a

T (K)	ρ (kg/m ³)	c_p (kJ/kg · K)	$\mu \cdot 10^7$ (N · s/m ²)	$\nu \cdot 10^6$ (m ² /s)	$k \cdot 10^3$ (W/m · K)	$\alpha \cdot 10^6$ (m ² /s)	Pr
Air							
100	3.5562	1.032	71.1	2.00	9.34	2.54	0.786
150	2.3364	1.012	103.4	4.426	13.8	5.84	0.758
200	1.7458	1.007	132.5	7.590	18.1	10.3	0.737
250	1.3947	1.006	159.6	11.44	22.3	15.9	0.720
300	1.1614	1.007	184.6	15.89	26.3	22.5	0.707
350	0.9950	1.009	208.2	20.92	30.0	29.9	0.700
400	0.8711	1.014	230.1	26.41	33.8	38.3	0.690
450	0.7740	1.021	250.7	32.39	37.3	47.2	0.686
500	0.6964	1.030	270.1	38.79	40.7	56.7	0.684
550	0.6329	1.040	288.4	45.57	43.9	66.7	0.683
600	0.5804	1.051	305.8	52.69	46.9	76.9	0.685
650	0.5356	1.063	322.5	60.21	49.7	87.3	0.690
700	0.4975	1.075	338.8	68.10	52.4	98.0	0.695
750	0.4643	1.087	354.6	76.37	54.9	109	0.702
800	0.4354	1.099	369.8	84.93	57.3	120	0.709
850	0.4097	1.110	384.3	93.80	59.6	131	0.716
900	0.3868	1.121	398.1	102.9	62.0	143	0.720
950	0.3666	1.131	411.3	112.2	64.3	155	0.723
1000	0.3482	1.141	424.4	121.9	66.7	168	0.726
1100	0.3166	1.159	449.0	141.8	71.5	195	0.728
1200	0.2902	1.175	473.0	162.9	76.3	224	0.728
1300	0.2679	1.189	496.0	185.1	82	238	0.719
1400	0.2488	1.207	530	213	91	303	0.703
1500	0.2322	1.230	557	240	100	350	0.685
1600	0.2177	1.248	584	268	106	390	0.688
1700	0.2049	1.267	611	298	113	435	0.685
1800	0.1935	1.286	637	329	120	482	0.683
1900	0.1833	1.307	663	362	128	534	0.677
2000	0.1741	1.337	689	396	137	589	0.672
2100	0.1658	1.372	715	431	147	646	0.667
2200	0.1582	1.417	740	468	160	714	0.655
2300	0.1513	1.478	766	506	175	783	0.647
2400	0.1448	1.558	792	547	196	869	0.630
2500	0.1389	1.665	818	589	222	960	0.613
3000	0.1135	2.726	955	841	486	1570	0.536
Ammonia (NH₃)							
300	0.6894	2.158	101.5	14.7	24.7	16.6	0.887
320	0.6448	2.170	109	16.9	27.2	19.4	0.870
340	0.6059	2.192	116.5	19.2	29.3	22.1	0.872
360	0.5716	2.221	124	21.7	31.6	24.9	0.872
380	0.5410	2.254	131	24.2	34.0	27.9	0.869

Appendix D: CALCULATION FOR CONVECTION COEFFICIENT

APPENDIX D - CALCULATIONS OF CONVECTION HEAT TRANSFER COEFFICIENT

Assumptions

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

Known information

Density of borosilicate glass, $\rho = 2230 \text{ kg/m}^3$

Specific heat capacity of borosilicate glass, $c = 835 \text{ J/kg} \cdot \text{K}$

Appendix D: CALCULATION FOR CONVECTION COEFFICIENT

Calculations for convection coefficients during annealing process (cool to 55°C)

Assumptions

Since the chip element is cooled by room temperature air through at laminar flow conditions (in the range of $< 5 \times 10^5$).

i.e. ambient air temperature, $T_\infty = 25^\circ\text{C} = 298\text{ K}$ (room temperature)

Assuming the surface temperature, T_s , has already attained the desired temperature for annealing process to take place.

i.e. surface temperature, $T_s = 55^\circ\text{C} = 328\text{ K}$

Obtaining the following data from Table 1 in Appendix C

All properties are evaluated at film temperature, $T_f = \frac{1}{2}(328+298) = 313\text{ K}$

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

kinematic viscosity, $\nu = 17.1978 \times 10^{-6}\text{ m}^2/\text{s}$

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

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

thermal diffusivity, $\alpha = 24.424 \times 10^{-6}\text{ m}^2/\text{s}$

thermal conductivity, $k = 27.262 \times 10^{-3}\text{ W/m.K}$

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

$$\text{Re}_L = \frac{VL_{\text{capillary}}}{\nu}$$

where V is the air flow velocity and $L_{\text{capillary}}$ is the length of each capillary .

Convection coefficient for top and bottom horizontal surface of cooled plate

$$\text{Re}_L = \frac{V(0.018)}{17.1978 \times 10^{-6}} = 1046.65V \quad (< 5 \times 10^5, \therefore \text{laminar flow})$$

$$\begin{aligned} \text{Nusselt number, } \overline{Nu}_L &= 0.664 \text{Re}_L^{1/2} \text{Pr}^{1/3} \\ &= 19.12V^{1/2} \end{aligned}$$

Appendix D: CALCULATION FOR CONVECTION COEFFICIENT

$$\begin{aligned}\text{Average convection coefficient, } \bar{h} &= \frac{\overline{Nu_x} \cdot k}{L} \\ &= 28.96 V^{1/2} \quad \left(\frac{W}{m^2 \cdot K} \right)\end{aligned}$$

$$\text{For incoming air velocity of 4 m/s, } \bar{h} = 58 \quad \left(\frac{W}{m^2 \cdot K} \right)$$

CoolPower
Solutions

- DC fans with electronically commutated external rotor motor. Fully integrated commutation electronics.
- With electronic protection against reverse polarity. The fan only operates when the polarity is correct. Impedance-protected against blocking and overloading.
- Fan of fibreglass reinforced plastic. PBT housing, PA impeller.
- Air exhaust over struts. Rotational direction CCW looking at rotor.
- Electrical connection via 2 leads AWG 28, TR 64. Stripped and tinned ends
- Mass 17 g (27 g).

SERIES 400F

40x40x10 mm

Air Flow	Air Flow	Nominal Voltage	Voltage Range	Noise	Sinter-Sleeve Bearings	Ball Bearings	Power Input	Nominal Speed	Temperature Range	Service Life L ₁₀		Curve	Type
m ³ /h	CFM	V DC	V DC	dB(A)	bels	⊙ / ●	Watt	min ⁻¹	°C	Hours	Hours		
8	4.7	5	4.5...5.5	26	4.5	⊙	0.7	5400	-20...+70	45000 / 15000		2	405 F
6	3.5	12	10...14	19	4.0	⊙	0.6	4300	-20...+70	45000 / 15000		1	412 FM
8	4.7	12	10...14	26	4.5	⊙	0.7	5400	-20...+70	45000 / 15000		2	412 F
9	5.3	12	10...14	29	4.9	⊙	0.8	6000	-20...+70	45000 / 15000		3	412 FH
8	4.7	24	20...28	26	4.5	⊙	0.8	5400	-20...+70	45000 / 15000		2	414 F

SERIES 400

40x40x20 mm

10	5.9	5	4.5...5.5	18	3.8	0.9	6000	-20...+70	50000 / 20000	4	405
10	5.9	12	10...14	18	3.8	1.0	6000	-20...+70	50000 / 20000	4	412
13.5	8.0	12	10...14	29	3.8	1.6	8100	-20...+60	45000 / 15000	5	412H
10	5.9	24	20...28	18	3.8	1.0	6000	-20...+70	50000 / 20000	4	414
13.5	8.0	24	20...26.5	29	4.7	1.6	8100	-20...+70	45000 / 15000	5	414H

Type 412H and 414H with electronic protection against blocking

Type	Dimension:	A	B	C
400F		10 ^{+0.3} _{-0.1}	Ø3.5 ±0.1	Ø3.5 ±0.1
400		20 ±0.15	Ø4.3 ±0.1	Ø4.3 ±0.1

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