Final Year Project Report

Precise temperature control for nucleic-acid amplification based microfluidic devices

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Abstract

The area of microfluidics is a multidisciplinary field focusing on the academic and commercial aspects of the behaviour of fluids at small volumes. By utilizing these unique physical properties both the speed and efficiency of many macro scale biological and chemical analysis can be greatly enhanced. This has led to the concept of a Lab on Chip (LOC). The premises of LOC are a sample in / answer out system integrated unto a single chip of relatively small dimensions. While the field itself has been in existence for over two decades, it has yet to make significant practical inroads beyond numerous academic articles and a handful of commercially successful products. One particular application of LOC is in the area of pathogen detection and in particular the analysis of Mastitis for use in the dairy industry. A portable, accurate and swift onsite analysis system would prevent the contamination of large volumes of milk at the source. An essential component of such a device is the PCR chamber. This chamber allows for DNA amplification to occur which in turns allows for the post PCR analysis to detect the presence of Mastitis DNA and hence identify contaminated milk at the source. By preventing this relatively small batch of milk from contaminating the greater supply then significant cost savings may be made. The purpose of this project was to investigate the physical parameters involved in heating / cooling designing aspects of such a PCR chamber.

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Chapter 1

1.1 Introduction

The term microfluidics itself refers to “the science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} liters) amounts of fluids, using channels with dimensions of tens to hundreds of micrometers.” [1] The origins of Microfluidics can be traced back to a paper by Purcell, Life at low Reynolds number [2], in which he outlined some of the unique physical properties of a fluid at a low Reynolds number (Re < 2300). When confined to such small channel dimensions viscous forces tend to dominate over inertial forces within the fluid resulting in a Laminar flow regime. This laminar flow regime has a major effect on the behaviour of the fluid by generally limiting the fluid to mixing by diffusion only. Hence it is possible to have several parallel streams of fluids flowing together within the same micro channel due to diffusion limited mixing. By varying the dimensions of the channel along with that of the laminar streams one can use diffusion for particle separation/sorting and various assays [3]. As the time (t) for a particle to diffuse within a channel is proportional to the square of the distance (d), then diffusion becomes an extremely efficient method of mixing at the microscale. This efficiency is essential as the viscous forces that allow for laminar flow preclude macroscale mixing such as convection. Additional effects at the microscale include a substantial increase in the surface area to volume ratio and an increase in the importance of surface tension forces [4] [5]. The review paper “PHYSICS AND APPLICATIONS OF MICROFLUIDICS IN BIOLOGY” [4] offer a gentle introduction to the subject while the review paper “Microfluidics: Fluid physics at the nanoliter scale” [5] offers a much more detailed look into the physics.

One of the first practical devices to employ microfluidic properties was a miniaturized gas chromatograph integrated onto a silicone substrate [6]. The manufacturing techniques used were derived from the integrated circuit fabrication industry. The result was an injection system that was three orders of magnitude smaller than anything commercially available at the time and capable of injecting sample pulse volumes down to one nano litter. However further microfluidics modules were merely components of more substantial devices. The mainstream concept of numerous fully integrated lab on a chip (LOC) devices or micro total analysis systems (μ-TAS) did not come into being until the early 90s [7][8]. The premise of LOC is a sample in/answer out system that is integrated unto a single chip of relatively small dimensions. Hence the LOC device can exploit any of the advantages of working at micro scales such as requiring lower sample/reagent volumes, faster analysis, minimized footprint and more cost effective fabrication. Such a LOC device designed for a particular analysis could compose of a sample input module, a sample preparation chamber, a reagent storage module, various reactors and mixer modules, a separation module, a detector module and power source that are all interconnected onto a single chip [9]. Figure 1.1 shows a diagrammatic representation of the
Precision and simplification of analysis can be achieved through both the removal of human error and the need for numerous individual analytical devices. Unfortunately LOC devices do however require novel fabrication and design techniques [10]. The need to integrate numerous microfluidic modules onto a single chip brings up issues with thermal isolation and connectivity between modules. Automation also becomes a major issue as traditional actuators or pumps are too bulky or become defunct at microscales. Reagent storage (high pressure or cryostorage) and sample preparation also adds to the difficulty of achieving a perfect sample in/answer out system.
Chapter 2

Theory

2.1 PCR

The polymerase chain reaction (PCR) is a highly effective method of DNA amplification. A polymerase is an enzyme whose function is the polymerisation of nucleic acids. In particular, a DNA polymerase is an enzyme involved in the polymerisation of deoxyribonucleotides of DNA strands. The most ubiquitous of the PCR polymerases is the Taq polymerase due to it being relatively thermostable. The PCR reaction itself can generally be described through three steps [12]. Initially, the DNA double helix is heated to approximately 95°C in order to disassociate the double helix into two complimentary separate strands; this process is also known as melting. Next, the temperature is decreased to between 50 and 65°C along with the addition of two types of primer. The primers being two specifically chosen strands of nucleic acid. Primers are required for use by the polymerase in order to replicate the initial DNA strands of interest. Only once a primer has attached itself to a DNA strand can polymerases begin to move along the DNA strand and allow replication. Hence the initial need for choosing primers capable of attaching to the complementary DNA strands of interest. In order to increase the probability of primer attachment, a high concentration of primers is required. This step is also known as annealing. The final step known as elongation involves raising the temperature to 72°C in order to allow the DNA polymerase to begin replication along the combined primer/DNA strands. Figures 2.1 and 2.2 below highlight the basic PCR technique.

Fig. 2.1 Graph of a single cycle in the PCR process. Image reproduced from reference [12]
Figure 2.1 above highlights the main steps of PCR cycling in terms of temperature change. While figure 2.2 gives a graphical description of the PCR process. These main PCR steps are repeated, with the amount of DNA replicated increasing exponentially with each cycle. In a standard laboratory environment PCR is achieved via a thermo-cycler. All the components of PCR such as buffers, primers, polymerases and the DNA sample can be mixed altogether throughout the PCR cycling process. This simplicity in design of the PCR procedure has even earned the inventor a noble prize. Prior to PCR many analysis of DNA required destruction of the initial sample or required the initial sample to be of a certain concentration in order to be analyzed.

In terms of PCR devices suitable for use in a LOC device there are examples of thermal convection PCR chambers with dimensions of 15 mm x 1.5 mm and even 5 mm x 0.5 mm. As the name suggests PCR is driven in this case by terminal convection through the use of a carefully modelled temperature gradient across modelled chamber geometry. Their resulting Thermal convection driven laminar flow between the regions of varying temperature profile emulates the principal of thermal cycling allowing PCR to be achieved.

There are however further difficulties, beyond dimensions, to the implementation of PCR on a LOC device. One such difficulty is the requirement of preconditioning of the PCR chamber (also known as passivation) by coating it in a PCR inert material. In addition sample processing such as cell capture, nucleic acid extraction and further purification is often necessary before PCR can take place. There may also be the requirement of the implementation of a real-time detector and also the standard post PCR analysis such as capillary gel electrophoresis on to the LOC device.
2.2 Heat transfer

The area of Heat transfer is concerned with physical mechanisms of heat movement. Heat itself is defined as not an actual form of energy but a process of energy transfer. However there is a form of energy associated with heat transfer and this is known as thermal energy. Thermal energy refers to the sensible and latent components of internal energy. These being translational, vibrational and rotational motion of atoms or molecules, the energy stored in chemical bonds between atoms and finally the binding forces in the nucleus. While thermodynamics does provide insight into the initial and final states of a system in after heating has occurred, it does not provide any insight into the dynamic process of heat transfer.

For this project only heat transfer of a system in terms of conduction is considered. In heat transfer there are two main types of heat transfer steady state and transient. In the transient phase there is a change in heat flux as a function of time through out the system and for steady state the heat flux at all locations remains constant. Note however the use of the term flux which the rate of thermal energy passing through a surface, hence for both types of heat transfer there is always a flow of energy.

From the first law of thermodynamics we can consider a control volume for a closed system for which the change in energy stored after over a time interval (Final time minus the initial time) can be described by the net heat transfer (Q) and the work done by the system (-W). Expressing the control surface idea in energy flow terms and on a rate basis gives equation 2.2.1.

\[ E_{in} - E_{out} + E_{st} = \frac{dE_t}{dt} \]

Equation 2.2.1

This states that at an instant that the rate of increase in energy with in the closed volume is the sum of the total energy flow in and the total energy generated internally minus the total energy flow out.

Heat transfer due to conduction occurs when ever there is a temperature difference between to objects or a temperature distribution within an object. This mechanism is described via the heat transfer equation also known as Fourier’s Law this is stated in equation 2.2.2 where \( q \) = Heat transfer rate (J/s or W), \( k \) = thermal conductivity (W/m.K) which is property of material itself and \( \frac{dT}{dx} \) is the temperature gradient.

Note the presence of the minus sign is required as heat transfer only occurs form the higher temperature region to the lower temperature. The heat flux equation can then be derived by dividing equation 2.2.2 by area (A). this yields equation 2.2.3 with \( q'' = \) Heat Flux (W/m2)
\[ q_x = ka \frac{\Delta T}{\Delta x} = -kA \frac{dT}{dx} \]

**Equation 2.2.2**

\[ q''_x = \frac{q_x}{A} = -k \frac{dT}{dx} = k \frac{\Delta T}{\Delta x} = k \frac{T_1 - T_2}{L} \]

**Equation 2.2.3**

One can define a differential control element in order to determine the temperature distribution. Using equation 2.2.1 as a template and substituting in various rate terms yields the Heat diffusion equation (equation 2.2.4).

\[
\frac{\partial}{\partial x} \left( k \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y} \left( k \frac{\partial T}{\partial y} \right) + \frac{\partial}{\partial z} \left( k \frac{\partial T}{\partial z} \right) + q^* = \rho c_p \frac{\partial T}{\partial t}
\]

**Equation 2.2.4**

The derivative terms on the right hand side of equation 2.2.4 relate back to the energy in rate and energy out rate terms in equation 2.2.1. The \( q^* \) relates back to the energy generation rate and the left hand side terms relate to the rate energy stored term in 2.2.1 but neglecting the latent effect due to no change of phase. Depending upon the energy transfer processes occurring within a material, the Heat equation can be simplified further. In the case constant thermal conductivity we simplify to equation 2.2.5, where \( \alpha \) is the thermal diffusivity (m²/s) property of material.

\[
\left( \frac{\partial^2 T}{\partial x^2} \right) + \left( \frac{\partial^2 T}{\partial y^2} \right) + \left( \frac{\partial^2 T}{\partial z^2} \right) + \frac{q^*}{k} = \frac{1}{\alpha} \frac{\partial T}{\partial t}
\]

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

**Equation 1.2.5**

In the case of steady state conduction there is no change in heat flux in time within a material only with position hence the elimination of the left hand side of the equation 2.2.6.
\[
\frac{\partial}{\partial x} \left( k \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y} \left( k \frac{\partial T}{\partial y} \right) + \frac{\partial}{\partial z} \left( k \frac{\partial T}{\partial z} \right) + q^* = 0
\]

Equation 2.2.6

One can now apply the heat equation to finding the temperature distribution and then conduction heat transfer rate for a 1-D steady state conductor as follows:

a. Solve Heat equation with Boundary conditions.

b. As in 1-D assume plane wall with no heat generation and that heat flux is independent of position (x). This allow the heat equation to be reduced to equation 2.2.7.

c. Integrate twice to get general solution and ally the boundary conditions to get the constants of integration

d. The yields equation 2.2.8 giving the temperature as a function of position (x)

\[
\frac{\partial}{\partial x} \left( k \frac{\partial T}{\partial x} \right) = 0
\]

Equation 2.2.7

\[
T(x) = (T_{r,2} - T_{r,1}) \frac{x}{L} + T_{r,1}
\]

Equation 2.2.8

An alternative way of analyzing steady state heat transfer in 1-D is in terms of thermal resistances which can be extended to the lump capacitance model for transient conduction. However finite element analysis (FEA) can be applied to heat transfer when dealing with conduction in 1-D, 2-D and 3-D. FEA works for both steady state and transient conduction equally well. There a many computational FEA modeler programmes available (such as Comsol)) which allow for quick and accurate solutions to heat transfer solutions

For an in-depth look at heat transfer please refer to Fundamentals of heat and mass transfer [14]
2.3 Labview

Laboratory virtual instrument engineering workbench (labVIEW) is a graphically based programming environment. This graphical programming is represented in the form of block diagrams, front panel user interfaces (also known more commonly as a graphical user interface (GUI)) and dataflow code execution. In addition to graphical programming there also exists data acquisition (DAQ) at the heart of LabVIEW's design. In order for the LabVIEW graphical environment to receive or output data a National Instruments DAQ board or chassis with I/O modules is required. LabVIEW's programming structure while fundamentally different from standard object orientated programming languages such as C++ does share a few programming structures and principles. For example a LabVIEW block diagram could be considered akin to a program code, a subVI an object and VI itself a program. LabVIEW programming also depends upon for loops and while loops in addition to being capable of Boolean operations. However the data flow execution dependents of LabVIEW means that direct conversion or comparison with other programming languages is not always possible.

Dataflow code execution basically means that functions can only execute after receiving their required input data. A LabVIEW program is known as a virtual instrument (VI). A VI is composed of a front panel user interface displaying and allowing the input and output of information for a user. The VI's actual code is represented by the block diagram. The block diagram contains all of the necessary functions and programming structures necessary for the operation of the VI. Itself can be transformed into a subVI and then used within another primary VI. In order for a sub VI to interact with the primary VI it requires connectors for data input and output to the VI. In addition such connectors can be used to transfer data between primary VIs. The sub VI itself is represented on the block diagram as a sub VI icon. Within the block diagram window terminals connect to complementing terminals on the front panel to allow the input and output of data between the two windows. Nodes allow for program execution elements such as operator and statements which exists in normal programming languages to be implemented within a VI. In particular there exists a type of node called a structure which behaves like loops or case statements allowing code within such a structure to be executed conditionally or repeatedly. The number of iterations of a structure can be controlled via the iteration thermal and timing may be achieved via a function from the time and dialogue sub pallet. Nodes and terminals are connected via wires. Which in essence carry data throughout the block diagram and allow for the creation of a dataflow sequential execution?

When considering data acquisition the path which data takes form the observable to the user GUI front panel is as follows: Transducer to I/O module to NI-DAQ chassis converted to usable information by the DAQ drivers and hardware, passed onto the labVIEW block diagram for processing and code execution and finally outputted for the user to see via the front panel. Depending on the requirement a particular I/O module can input or output digital or analogue signals. On a block diagram level each individual input or output on an I/O module is represented by a channel and in this way can interact with the user at a programme level.
In addition to all the programming identities discussed above LabView provides countless additional structures, functions, nodes, subVIs available through the various sub palettes from the labview programme menu. This in addition to allow control of I/O module channels from a subVI or DAQ-Driver level makes LabView a powerful data acquisition, analysis and modelling tool. However it is this level of varying control which can also overwhelm a novice not just through its varying levels of complexity but also through its vast offering of choice.

For a in-depth look at labVIEW programming please refer to LabVIEW graphical programming : practical applications in instrumentation and control [15].

2.4 RTD

A resistance temperature detector (RTD) is a highly accurate electrical temperature measurement instrument with even industrial standard RTDs providing accuracy up to +/-0.1°C. More expensive Standard platinum resistance thermometers (SPRTs) reaching accuracy up to +/-0.0001°C are also commercially available. There are two basic types of RTD with one being composed of a thin wire coil element of high purity metal. The other is known as a thin film RTD and is generally composed of a thin film of platinum deposited onto a ceramic substrate. RTDs operate on the principle of varying resistance of the metal element with temperature and can be used as passive sensors but more accurately with the addition of a small current. The resistance increases linearly with temperature within the useful temperature range of the RTD

The variation in resistance with temperature can be described via the Calendar-Van Dusen equation.

\[ R_T = R_0 \left[ 1 + AT + B T^2 + C T^3 (T - 100) \right] \quad (-290 \, ^\circ \text{C} < T < 0 \, ^\circ \text{C}) \]

\[ R_T = R_0 \left[ 1 + AT + B T^2 \right] \quad (0 \, ^\circ \text{C} \leq T < 850 \, ^\circ \text{C}). \]

With \( R_T \) being the measured resistance at a particular temperature, \( R_0 \) being the RTDs resistance at 0°C. The coefficients A, B and C are unique to the particular RTD sensor being used. In general the coefficient A is many orders of magnitude greater than either B or C and hence the linear relationship between resistance and temperature for an RTD. Figure 2.4 below displays some typical coil and thin-film RTD's.
Chapter 3
Experimental

3.1 Scaled up Model of PCR chamber

The main part of the experiment was to build and test a scaled up model of a PCR chamber as shown in figure 3.1 below. Oil was chosen as an acceptable liquid to test with as it did not undergo significant evaporation during experimentation. The oil used was household sunflower oil. The heater used was a commercial grade polyimide insulated etched foil heater (Minco HK5950). This type of heater was chosen, as it possessed excellent heating efficiency and thermal uniformity potential in addition to a satisfactory cooling efficiency. The heater was adhered to the bottom of a standard glass slide and a chamber of Polydimethylsiloxane (PDMS) was manufactured to enclose the oil. The sidewalls of each chamber were formed from a single piece of punched PDMS. The top cover where necessary was also formed from a single piece of PDMS. PDMS was chosen due to it being an extremely versatile material that had a long proven history of use in the manufacture of microfluidic devices. The temperature sensor chosen was a flat film platinum resistance temperature detector (Labfacility Pt100 DM-508). An RTD was chosen, over a thermistor, due to its relatively linear resistance versus temperature relationship. The RTD used yielded
an optimal accuracy of +/- 0.15 degrees at 0 degrees Celsius and dropped to +/- 0.35 at 100 degrees.

Fig 3.1 Diagram showing scaled up model with Dimensions.

After having the chamber assembled it was then necessary to implement a method of control via LabView. Ideally labview would have been required to read the resistance of the RTD, use the Callendar-Van Dusen equation to obtain a temperature measurement. Taking this measurement labview would then have dynamically varied the voltage to the heater and the speed of a fan in order to control the temperature of the oil in the chamber. However due to limited time it was not possible to implement this design fully.

3.2 Implementation:

3.2.1 Basic test:

For an initial test of concept the heater was connected directly to the PCU and a thermocouple connected to a multimeter was used to measure temperature. A chamber made from punched out PDMS was glued to a glass slide and heater adhered on the opposite side of the glass slide. The chamber was filled with cooking oil. A dataset of temperature versus time for the oil within the chamber was recorded for a series of increasing voltages applied to the heater. Once graphed the datasets showed clearly that an increase in voltage applied to the heater result in a faster increase in temperature and a higher final temperature.

3.2.2 Primary experimental layout and technique:

1. Three individual chambers of varying volumes and dimensions are manufactured: well A, well B and well C. For well A the external PDMS dimensions were 50.2 mm x 2.5 mm and internal dimensions of 39.5 mm x 12.5 mm which is a depth of 4.2 mm. Well B the external dimensions were 90 mm x 20 mm and internal dimensions of 10 mm x 10 mm with depth of 4.2 mm. For well C external dimensions were 90 mm x 20 mm and internal
dimensions of 10 mm x 10 mm and a total depth of 7.7 mm. The dimensions of the glass slide for all wells was 1 mm x 26 mm x 70 mm.

2. A heater was adhered to the bottom of each glass slide and each well was glued to each glass slide with a very thin layer of epoxy resin.

3. The heater and RTD were wired up as shown in fig x and the circuit layout is shown in fig y below.

![Fig 3.1.1](image1.png)

![Fig 3.1.2](image2.png)

4. A Labview programme was written which allowed control of the heater via PWM and for the control of and recording of reading from the RTD.

5. Individually each well was filled with a volume of sunflower oil. the first experiment involved well A filled with 700 micro liters, the second experiment involved well B with 400 micro liters , the third experiment involved well C with 700 micro liters and the 4th and final experiment involved Well A with 1400 micro liters of sunflower oil.
6. The RTD was placed in as centre a position as possible with in the oil. For each well the temperature change with time was recorded for a PWM voltage of 4 V, 8V and 12V with each reading taken 3 times. The average of all 3 runs at each voltage was graphed along with the standard deviation.

3.3 LabVIEW Program

3.3.1 Pulse Width Modulation (PWM) control of heater

PWM is a technique whereby power is delivered to an analogue circuit via a Digital pulse train. By varying the duty cycle of a pulse train with a certain voltage, an analogue component such as a Light bulb will behave as if it is receiving a fraction of the actual peak voltage. This holds provided that the frequency remains constant and the cycle period is relatively short compared to the load's response time to a change in the voltage state (on/off).

![PWM Waveforms](image.png)

Fig 3.3.1 [17]

Considering the 3 different duty cycles above when 50% the voltage is at 5V (on) for half one period and 0V (off) for the other half, hence producing an average voltage of 2.5V. At 90% duty cycle the voltage is on for 9/10 of one period and off for 1/10 thus producing an average voltage of 4.5V.
In order to achieve PWM voltage control over the heater I created a VI which produced a continuous square wave with voltage peaks between 0 and 5 V. The PWM pulse train could be modified via the Duty Cycle or Frequency, and responded to changes in approximately a second.

However simply outputting a square wave directly set at 5V did not produce the desired pulse train as the voltage would alternate between +/- 5 V. In order to create the necessary 0-5 V output the waveform had to be set to half the desired maximum frequency and offset by half the desired maximum frequency. Hence the need for a Divide function after the voltage output to allow the user to simply input the desired maximum voltage.

From the block diagram above the outer box represent structure which can be time controlled by the wait until next millisecond multiple time dialogue function.
3.3.2 PWM control of heater integrated with automated recording of time vs. temperature readings for dual RTDs

The VI font panel and block diagram below describe the final VI which allow for both control of the heater via PWM and the recording of readings from two RTDs simultaneously. In addition the VI automatically passes each of the RTD reading to an output file. Unlike the previous VI for PWM control only this VI has been built from high level subVIs provided by labview. Note form the front panels how the high level subVIs automatically controls the RTD signal current level for a verity of RTD types. In this case as the RTD is a Pt100 (a platinum flat film type with a resistance of 100 ohms at 0 degrees Celsius. The resistance type is set to 3-wires, even though the Pt100 RTD is a two wire type, in the front panel as the RTD DAQ I/O module only allow for this type. A three wire type RTD is considered more accurate as the presence of a third wire on the RTD allow for compensation for errors due to the minute thermal heating of the RTD due to the signal current. By simply connecting one of the RTD wires of the Pt100 RTD to two of the three inputs on the RTD DAQ I/O module and the other wire to the single remaining input, measurements using the two wire RTD can be obtained. However in this case the compensation for error due to thermal heating is no longer as efficient.

![Block Diagram](image)

Fig 3.3.4

Looking at the block diagram the main subVI of interest outside of the loop structure is the AI TEMP RTD subVI. As described above it can automatically input the necessary A, B and C constants into the calendar-van dusen equation and then output
the actual temperature for whatever input resistance it receives for the designated physical channel or in the case of this particular setup channels. All that is required on the user’s part is to identify the RTD type, the resistance configuration along with the max and min temperature values expected during the reading. It then simply outputs the temperature value for use in the next relevant VI. The next subVI along the dataflow path is the SAMPLE CLOCK VI which takes in the continuous flow of temperature data and outputs the temperature data from both channels (one for each RTD) at a rate in Hertz which can be defined by the user on the front panel.

The subVI just before the structure is called the START subVI and this subVI along with the CLEAR TASK subVI, at the opposite end of the structure, are only required as programming formalities appearing when ever the SAMPLE Clock subVI is used. Finally the POP UP DIALOG BOX subVI is there to display any error that may occur in the running of this VI. In order for the user to stop acquiring waveform data (in this case time vs. temperature) the loop can be stopped via the stop button on the front panel.

With in the loop structure each of the ANALOG 1D WFM subVIs takes the sampled data coming from outside the loop at a rate defined by the ‘samples to be read’ value. For example if the sample clock rate is set to 10 Hz then a “samples to be read” value of 10 would allow the ANALOG 1D WFM subVI to sample the same number of times a second (in this case ten samples a second or one sample every millisecond for both RTD channels, However for the bottom ANALOG 1D WFM subVI with in the loop structure with its “samples to be read” value set to 1 can only output one out of every ten of the initial samples and hence only outputs one sample a second for both RTD channels. Hence the presence of four waveform graphs on the front panel. The time vs. temperature waveform samples for both RTDs at both sample rates are also simultaneously outputted to individual files to allow for further analysis by the user.

Fig 3.3.5
3.4 COMSOL MODEL

The comsol modelling environment for heat transfer for Well A required the input of the specific heat capacities of the PDMS, Glass slide, and sunflower oil in addition to the thermal generation properties of the heater element. The comsol modelling environment also requires that all dimensions of every element is entered. While the specific heat capacities for the glass and PDMS were easily obtained, the specific heat capacity for sunflower oil changes as a function of temperature. This was determined by graphing the data of table 2 from ref 25, then extending the curve back to 20 degrees Celsius and finally taking the average heat capacity value for the temperature range already found from the previous physical experiment with Well A. The boundary conditions were entered for a system composed of a transient heat generating element and for an air temp of 20 degrees Celsius. Finally the Well A was modelled for a heater voltage of 4V, 8V and 12V.

Chapter 4

Results and Discussion

4.1 EXPERIMENTAL RESULTS

The results of the 4 main experiments are shown below:
Fig. 4.1.1 Well A with 1400 micro-liters of sunflower oil displaying the time vs. temperature reading for heater voltages of 4V, 8V and 12 V

Fig. 4.1.2 Well A with 700 micro-liters of sunflower oil displaying the time vs. temperature reading for heater voltages of 4, 8 and 12 V
4.1.1 Properties common to all experimental graphs

The most obvious common trend among all the graphs is the fact that the slope of each individual curve with in each graph increases with an increase in the voltage applied to the heater element. Also the final temperature reached by an individual curve relative to the others on the same graph increases as the voltage applied to the heater increases. These observations both make sense as the higher the voltage the higher the heat flux resulting in a faster heating time and a higher final temperature.

There are also important trends associated with each and every individual curve. There is initially a period of high increase in slope of the curve and then gradually it changes to an almost linear shaped curve. The linear region is associated with the Steady state heat transfer stage in which the heat flux at any point with in the well is a constant. There is still a transfer of heat energy from the heater through the oil and onto the air however the system has approaching its equilibrium position. The initial region of increasing slope is associated with the transient conduction phase and over
this region of time the heat flux at any particular region of the well varies with time. In particular the heat flux increases with time for a given position.

4.1.2 Effect of change in oil volume for Well A

When comparing two identical wells with differing volumes of oil we can see a general over all steeper slope in the transient regions and higher temperature in the linear region for a curve in the well with less volume. The curve from the well with the smaller volume also enters the linear stage earlier than for the same curve in the well with the greater volume of oil. This is most pronounced in the higher voltage curves such as the 8V and 12V. This behaviour makes sense in terms of heat transfer theory as less volume means for the same heat capacity oil that rate of increase in heat transfer would be faster.

4.1.3 Effect of identical oil volumes in different wells A and C

For a curve from well C there is an obvious smoothening of the increase in the slope as it moves towards the linear region when compared to the corresponding slope for Well A. This can be explained by the different construction of the two wells. Well A has a larger liquid surface area in contact with the heating element when compared to well C and hence there is a faster increase in heat flux for well A. the larger area of that generation also allow for well A to reach a higher temperature than that of well C in the same time period. However both Wells to have identical liquid surface volumes in contact with both the source the heat and the air thus the differences in the corresponding curves for the two wells must be dominated by heat generation effects.

4.1.4 Comparison of Wells B and C

Well B has a total oil volume of 400 micro-liters and well C a total oil volume of 700 micro- liters. During the transient conduction phase Well B displays a somewhat greater increase in slope compared to well C. However both curves tend increasingly towards converging in the linear phase. The heat generation area is identical in both and while well C has a greater total volume of oil it also has a smaller surface area in relation to its volume in contact with the air. The effect of the lower oil volume of cell B results in a much steeper slope and higher temperature during the transient heat transfer phase. The difference in temperature between corresponding curves in both wells decreases as both being to enter the steady state phase as well C has less surface area in contact with the air it is thus able to increase in temperature.
4.2 Comsol Model results

The results of the comsol model of well A are shown below

![Comsol Model Well A with 1400 micro-Litres](image)

Fig. 4.2.1 Comsol model of Well A displaying the modelled time vs. temperature reading at a point at the very centre of the modelled well for heater voltages of 4, 8 and 12 V

4.3 Comparison of model with experimental data

A comparison between the comsol model and the experimental data is shown below:
Fig. 4.3.1 Comparison of time vs. temperature readings at the centre point of well A for experimental Data and Comsol data. This data is displayed for heater voltages of 4V, 8V and 12V

The experimental results while having an overall lower temperature than the comsol model was successful. Both the model and experimental results displayed the properties of steady state heat transfer and transient heat transfer. The variation between the two graphs could be explained by firstly the fact that a constant average specific heat capacity for the sunflower oil was used in the model and the model. Secondly the RTD may have not been at the actual centre of the experimental well hence giving a slightly different profile. This would have more than likely to have been a position just above the centre.

The final 3 images below are of 2-D steady state temperature distribution slices taken at the centre of the 3-D models for 4V, 8V and 8V
Fig. 4.3.2: 2-D colour contrast model of the variation in temperature throughout Well A for a heater voltage of 4V for steady state conduction.

Fig. 4.3.3: 2-D colour contrast model of the variation in temperature throughout Well A for a heater voltage of 8V for steady state conduction.
Fig. 4.3.4 2-D colour contrast model of the variation in temperature throughout Well A for a heater voltage of 12V for steady state conduction.
Chapter 5

Conclusion

5.1 Final Conclusions

the following goals were successfully achieved:

(a) Designed and implemented a software interface (LabView) for temp sensor monitor and heater control.

(b) Used an electronic card to control sensor and heater.

(c) Understood and develop heat transfer model for the microfluidic chip which would allow the optimization of future generation of microfluidic chips that require thermal control.

For the most part the experimental data agreed with the basic theory of heat transfer. As did the comsol model with the experimentation. However the use of sunflower oil which has such a variable specific heat capacity was a mistake and glycerol would be a much better alternative for future experiments.

In general the actual design of thermal connection PCR chambers is highly computer intensive and I came up with for my project may prove difficult to implement due to the space premium on a LOC device. However I did find an alternative type of PCR chamber (11). This is known as convective polymerase chain reaction around micro immersion heater. This design seems to greatly simply both the PCR chamber design and temperature control regime.

Bibliography


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