ABSTRACT

KUNDURU, VINDHYA. Microcalorimetric Biosensors for DNA Melting Curve Analysis. (Under the direction of Glenn M Walker.)

Microcalorimetry enables the study of the thermodynamic properties of solid materials such as polymers and metal thin films. Recent advancements in microfabrication techniques and material science has extended the use of microcalorimetry to investigate thermodynamically driven reactions in biological macromolecules suspended in a fluid medium. Microcalorimetry enables direct measurement of small temperature or energy changes associated with the reaction within minuscule sample volumes.

In this work we exploit the fundamental principles of calorimetry and amalgamate novel silicon microfabrication techniques with custom designed capture electronics to create highly sensitive portable biosensors with sub-microliter resolution. The thesis lays the foundation for strategic enhancement of the design features through a guided evolution process. The performance metrics and limitations of each device generation were used as the key determinants for progressive renovation. We illustrate the design methodology and characterization process of developing microcalorimeters for measuring the heat of the reaction of biological liquid samples while the device is freely suspended in air without external insulation. The microcalorimeters thus developed successfully detected enthalpy changes in the order of 2$\mu$J with a sample consumption of 0.4$\mu$L and an average response time of 3.5 min.

Melting curve analysis (MCA) was selected as the pertinent application to demonstrate the performance of all device generations. MCA is a popular method for studying thermal characteristics of nucleic acids. DNA melting curve analysis primarily involves heating a fluorescently tagged mixture of double-stranded DNA (dsDNA) for detecting the melting temperature of dsDNA when it dissociates into its component single-stranded DNA (ssDNA). The shape and position of melting curves thus obtained suggest information about the length, specific heat capacity and relative base pair content of the DNA sample. Commercial MCA systems require sample volumes of about 150$\mu$L with typical sample concentrations of 0.2 mg/ml along with a measurement time on the order of 15 – 90 min.

All generations of our microcalorimetric devices consisted of a thin film heater and thermometer integrated on a common substrate to perform heating and sensing functions. Finite element modeling of the thin film microelectrodes and bioreactor chamber enabled optimized designs for uniform temperature distribution. The first generation glass-based devices had the capability to circumvent disadvantages such as evaporation and bubble-formation during conventional heating processes on microfluidic chips through a novel step-wise pressurization technique. The subsequent designs incorporated droplet samples encapsulated by oil to control...
evaporation. Wax, egg-white protein lysozyme, salmon testes DNA and purified genomic DNA with known melting temperatures were used as melting models to successfully demonstrate melting curve analysis.
Microcalorimetric Biosensors for DNA Melting Curve Analysis

by
Vindhya Kunduru

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

BioMedical Engineering
Raleigh, North Carolina
2014

APPROVED BY:

Nancy Allbritton
Greg McCarty

Michael Ramsey
Karen Week

Glenn M Walker
Chair of Advisory Committee
DEDICATION

To my mother Savitri, father Raja Reddy and husband Anand.
ACKNOWLEDGMENTS

I consider myself very fortunate to have had the opportunity to work with Glenn Walker. Glenn has been an excellent research adviser, teacher, a captivating orator and an inquisitive scientist with an eye for detail which was often intimidating. I express my deepest gratitude to him for guiding me through this research and helping me understand its many novel facets. My fondest memories are all those hours of discussions and white-board graffiti for trouble-shooting various aspects of my research. They elicited critical thinking and the ability to associate results with solid scientific rationale. I thank him for his support, constant encouragement and ample patience through all my years at NCSU.

I would like to thank my committee members Dr. Nancy Allbritton, Dr. Greg McCarty, Dr. Michael Ramsey and Dr. Karen Weck for helping me augment my research by providing their invaluable expertise and advice. I thank Dr. Michael Leming for graciously accepting the role of graduate school representative. I thank Dr. Shawn Gomez for signing off on my endless paperwork with the utmost grace. I thank Dr. John Muth and Joe Matthews from the ECE department for letting me use the E-Beam evaporator. A special thanks to Joe for patiently accommodating my incessant metal deposition needs at the shortest notice. I thank the in-house know-it-all guy Steve Callender for letting me pick his brains for help with projects of all sizes and shapes.

I thank Rekha Balasubramanyam, Stephanie Gootnick and Vilma Berg for expertly handling my often mindless inquiries with patience and their dedication to solving all my problems big and small with a smile. For keeping me in good spirits, pointing me away from disappointments and for helping me maintain sanity throughout, I thank my friends Alison Amos, Miguel Acosta, Sofie Permana, Stephanie Teeter, Peter Sotory and Adam Dengler. I thank Amy McPherson for helping me transition into the Tiny Biotools Lab with ease. An incredibly talented electrical engineer with an eye for creative design - Jeffrey Soohoo was immensely helpful when it came to trouble-shooting my noisy circuits. I thank him for all his help during my stay at NCSU. I thank Sita Lakkaraju my friend and co-founder of Mlinzi Vaccines LLC for sharing the onus of running a startup and efficiently taking charge of all responsibilities onto herself during my hibernation for completing this dissertation.

I want to thank my friends Aparna Hemadri, Indraneeal Takkilapati and Rama Gudavalli for being my faithful buddies since graduate school. Sunil Baliga and Karthik Sivaramakrishnan for being so resourceful and generous. My good old friends Smitha Chakravarthy and Divya Jagdish for always knowing the right things to say to help me out with dilemmas. Needless to say I want to thank the high-heeled horses Vanitha Viswanurthy, Seema Aman and Roopa Ramesh; Prem Kumar Maloo and Nitesh Rathod for helping me re-live good old college days.
during my visits back home. I thank my friend Chandrasekhar Annavarapu for his incredible company all through my stay in Durham and for seamlessly blending in to our lives much like family. Deepti and Kumar Reddi and all friends from Duke for making life seem like a deluge of cakes and fun!

I thank my parents-in-law for their unconditional love and support. I especially thank my dear doting mother-in-law Indira for her unwavering faith in me. Her sudden and premature demise created a void in the family leaving a long to-do list permanently unchecked. I miss her deeply. My heartfelt gratitude for my big brother Sagar and sister-in-law Nipa for their constant support and encouragement throughout my graduate student life. I also thank them for my nieces Aranya and Inika who have never failed to lift my spirits in the direst situations. I thank my younger brother Bhargav for being a protective and watchful guardian. I thank my dad Raja Reddy for motivating me with his insatiable thirst for knowledge and his power to question ceaselessly. My dear mother Savitri for making me the woman I am today. Her undying love and countless sacrifices have cushioned my very existence and will forever keep me in debt. Finally I thank the love of my life Anand for being the powerhouse of my confidence and inspiration. With his abysmal supply of motivation, fierce support and insightful criticism he always made it seem like I can achieve anything I put my mind to. I thank him for all those enlightening discussions on finite element modeling and thermodynamics. I look forward to life together with my best friend and flatterer Anand.
# TABLE OF CONTENTS

## LIST OF TABLES

<table>
<thead>
<tr>
<th>#</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>ix</td>
<td>ix</td>
</tr>
</tbody>
</table>

## LIST OF FIGURES

<table>
<thead>
<tr>
<th>#</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

## Chapter 1 Introduction

1.1 Purpose and Significance ........................................... 1
1.2 DNA .................................................................................. 2
   1.2.1 Background ................................................................. 2
   1.2.2 DNA thermodynamics .................................................. 3
1.3 Melting Curve Analysis .................................................. 5
   1.3.1 UV absorbance melting curves .................................... 5
   1.3.2 Fluorescence melting curves ....................................... 6
   1.3.3 Review: Current research ........................................ 7
1.4 Calorimetry ......................................................................... 8
   1.4.1 Calorimetry as a sensing tool .................................... 8
   1.4.2 Evolution of chip calorimetry .................................... 8
   1.4.3 Current research: Chip calorimetry ............................. 9
   1.4.4 Advantages of current design .................................... 10
   1.4.5 Applications in life sciences .................................... 11
1.5 Types of Thermal Analysis Techniques ................................ 12
1.6 Differential Thermal Analysis .......................................... 13
   1.6.1 Principle of operation .............................................. 13
   1.6.2 Applications .............................................................. 15
1.7 Differential Scanning Calorimetry ..................................... 15
   1.7.1 Principle of operation .............................................. 15
   1.7.2 DSC capabilities ..................................................... 15
1.8 Heat Transfer ...................................................................... 16
   1.8.1 Conduction .............................................................. 17
   1.8.2 Convection .............................................................. 17
   1.8.3 Transient heat transfer analysis .................................. 17
   1.8.4 Biot number (Bi) ..................................................... 18
   1.8.5 Lumped analysis ..................................................... 18
   1.8.6 Estimation of thermal capacitance of device ................. 19

## Chapter 2 Design Considerations

2.1 Thin Film Heater/Sensor Design ....................................... 21
   2.1.1 Evolution of thin film technology in microsystems .......... 21
   2.1.2 Types of thermal sensors ........................................... 22
   2.1.3 RTD: Sensing principle .............................................. 23
   2.1.4 Material selection .................................................... 25
2.2 Heater and RTD Design: Finite Element Analysis .................... 26
2.3 Heater Design .................................................................... 26
Chapter 3 Generation I: Glass-PDMS Hybrid

3.1 Background ........................................................................... 36
  3.1.1 Theoretical estimation of thermal time constant ............... 37
3.2 Bubble Formation and Evaporation ........................................ 37
  3.2.1 Bubbles during PCR ....................................................... 38
  3.2.2 Bubbles during MCA ..................................................... 38
3.3 Materials and Methods .......................................................... 39
  3.3.1 Experimental setup ........................................................ 39
  3.3.2 Buffers and reagents ....................................................... 41
  3.3.3 Fluorescence data analysis ............................................. 42
  3.3.4 Device fabrication ........................................................ 43
3.4 Results .................................................................................. 47
  3.4.1 RTD performance .......................................................... 47
  3.4.2 Thermal time constant ................................................. 48
  3.4.3 Demonstration of DNA melting ................................... 49
3.5 Discussion .............................................................................. 51
  3.5.1 Eliminating bubble formation ....................................... 51
  3.5.2 Nanoliter DNA injection volumes .................................. 51
3.6 Conclusions ........................................................................... 53

Chapter 4 Generation II: Wheatstone Bridge Based Glass-PDMS Hybrid

4.1 Wheatstone Bridge Circuitry .................................................. 54
  4.1.1 Background and applications ....................................... 55
4.2 Theory and Operation ............................................................. 55
  4.2.1 Modes of operation ....................................................... 56
  4.2.2 Linearizing wheatstone bridge output ......................... 57
4.3 Instrumentation Overview ...................................................... 59
  4.3.1 Operational amplifiers ............................................... 59
  4.3.2 Buffer amplifiers ......................................................... 59
  4.3.3 Differential amplifiers ............................................... 60
  4.3.4 Instrumentation amplifiers ....................................... 60
4.4 Materials and Methods .......................................................... 60
  4.4.1 Experimental setup ..................................................... 60
  4.4.2 Device fabrication ....................................................... 61
  4.4.3 Circuit components ..................................................... 62
  4.4.4 Operation methodology ............................................ 63
4.5 Results .................................................................................. 63
  4.5.1 Bridge response: control ............................................ 63
  4.5.2 Bridge response: Wax melting .................................... 65
4.6 Discussion .............................................................................. 66
Chapter 5 Generation III: Film Device for Droplet-based Detection .......................... 68
5.1 Electronic Instrumentation Overview ................................................................. 68
5.1.1 Wheatstone bridge: AC excitation ................................................................. 68
5.1.2 Lock-in amplifier ............................................................................................. 70
5.1.3 RC filters .......................................................................................................... 71
5.2 Background: Materials ....................................................................................... 72
5.2.1 Ferric chloride etching of copper ................................................................. 72
5.2.2 Polytetrafluoroethylene .................................................................................. 73
5.2.3 Glycerin ............................................................................................................ 73
5.2.4 Salmon DNA .................................................................................................... 73
5.3 Materials and Methods ....................................................................................... 74
5.3.1 Experimental setup .......................................................................................... 74
5.3.2 Buffers and reagents ....................................................................................... 76
5.3.3 Device fabrication ............................................................................................ 76
5.3.4 Droplet application ......................................................................................... 78
5.4 Results ................................................................................................................... 79
5.4.1 DNA fluorescence validation ......................................................................... 79
5.4.2 Demonstration of wax melting ....................................................................... 81
5.4.3 Demonstration of salmon DNA melting ......................................................... 83
5.5 Discussion and Conclusions ................................................................................ 85
5.5.1 Wax: Associated temperature changes ......................................................... 85
5.5.2 Conclusion ......................................................................................................... 85
5.5.3 Limitations of current design ........................................................................... 85
Chapter 6 Generation IV: Double Droplet Differential Detection ............................... 86
6.1 Background ........................................................................................................... 86
6.1.1 Thermal equilibrators ..................................................................................... 86
6.2 Materials and Methods ....................................................................................... 87
6.2.1 Experimental setup ....................................................................................... 87
6.2.2 Buffers and reagents ..................................................................................... 88
6.2.3 Device fabrication ........................................................................................... 89
6.2.4 Heater input: Constant power generation ..................................................... 90
6.2.5 Fluorescence validation experiments ............................................................. 92
6.3 Results ................................................................................................................... 92
6.3.1 Constant power source .................................................................................. 92
6.3.2 DNA melting: Fluorescence validation ........................................................ 93
6.3.3 Demonstration of wax melting ....................................................................... 93
6.3.4 Demonstration of salmon DNA melting ......................................................... 96
6.4 Discussion and Conclusions ................................................................................ 97
6.4.1 Wax melting ..................................................................................................... 97
6.4.2 Experimental determination of $Q$ ................................................................. 97
6.4.3 Conclusions ..................................................................................................... 98
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table Number</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Nearest-Neighbor Nucleotide Enthalpy Chart</td>
<td>5</td>
</tr>
<tr>
<td>Table 1.2</td>
<td>Common Thermal Analysis Techniques</td>
<td>12</td>
</tr>
<tr>
<td>Table 2.1</td>
<td>Comparison of Temperature Sensors</td>
<td>23</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Temperature Coefficient of Resistance ( \alpha(%/{ }^\circ C) ) of Common RTD Metals</td>
<td>25</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Device Construction Materials and Their Properties</td>
<td>27</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Water: Thermal and Material Parameters</td>
<td>33</td>
</tr>
<tr>
<td>Table 5.1</td>
<td>Device parameters</td>
<td>81</td>
</tr>
<tr>
<td>Table 5.2</td>
<td>Temperature mapping</td>
<td>81</td>
</tr>
<tr>
<td>Table 5.3</td>
<td>Device parameters</td>
<td>83</td>
</tr>
<tr>
<td>Table 5.4</td>
<td>Temperature mapping</td>
<td>83</td>
</tr>
<tr>
<td>Table 7.1</td>
<td>Thermal constants of device</td>
<td>114</td>
</tr>
<tr>
<td>Table 8.1</td>
<td>Performance Metrics</td>
<td>117</td>
</tr>
<tr>
<td>Table A.1</td>
<td>Thermal Properties of Materials Used</td>
<td>145</td>
</tr>
<tr>
<td>Figure Reference</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------</td>
<td>------</td>
</tr>
<tr>
<td>Figure 1.1</td>
<td>Method for predicting total transition enthalpy for DNA oligomer</td>
<td>5</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Fluorescence melting curve analysis</td>
<td>7</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Micromachined chip calorimeters</td>
<td>9</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Chip calorimeters with membrane components</td>
<td>10</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>DTA operation principle</td>
<td>14</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Typical DTA thermogram</td>
<td>14</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>DSC: Principle of operation</td>
<td>16</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Heat transfer model: Lumped analysis</td>
<td>19</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>RTD transfer function</td>
<td>24</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Heater geometry</td>
<td>27</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Heater temperature distribution</td>
<td>28</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Optimal RTD position with reference to heater</td>
<td>29</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Optimal RTD proximity to heater</td>
<td>30</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Optimal heater - RTD positioning</td>
<td>31</td>
</tr>
<tr>
<td>Figure 2.7</td>
<td>Marangoni effect</td>
<td>32</td>
</tr>
<tr>
<td>Figure 2.8</td>
<td>Optimal bioreactor geometry</td>
<td>33</td>
</tr>
<tr>
<td>Figure 2.9</td>
<td>Optimal bioreactor geometry</td>
<td>34</td>
</tr>
<tr>
<td>Figure 2.10</td>
<td>Comparative chamber geometries</td>
<td>34</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>System-level block diagram</td>
<td>39</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Acrylic jig for mounting glass-PDMS chip</td>
<td>40</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Mechanism of EvaGreen dye</td>
<td>42</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>Process flow for microelectrode fabrication</td>
<td>44</td>
</tr>
<tr>
<td>Figure 3.5</td>
<td>Process flow for PDMS microfluidics fabrication</td>
<td>45</td>
</tr>
<tr>
<td>Figure 3.6</td>
<td>Optical micrograph of device</td>
<td>46</td>
</tr>
<tr>
<td>Figure 3.7</td>
<td>Device overview in 3D</td>
<td>46</td>
</tr>
<tr>
<td>Figure 3.8</td>
<td>RTD calibration</td>
<td>47</td>
</tr>
<tr>
<td>Figure 3.9</td>
<td>Time constant of PDMS reaction chamber</td>
<td>48</td>
</tr>
<tr>
<td>Figure 3.10</td>
<td>Optical melting curves of dsDNA</td>
<td>50</td>
</tr>
<tr>
<td>Figure 3.11</td>
<td>Bubble formation inside PDMS microreactors</td>
<td>51</td>
</tr>
<tr>
<td>Figure 3.12</td>
<td>Nanoliter DNA injection volumes</td>
<td>52</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Typical Wheatstone bridge circuit</td>
<td>55</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Non-linear wheatstone bridge output voltage</td>
<td>57</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>Wheatstone bridge: Sensing mechanism</td>
<td>58</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>Types of opamp circuits</td>
<td>61</td>
</tr>
<tr>
<td>Figure 4.5</td>
<td>Block diagram of experimental setup</td>
<td>61</td>
</tr>
<tr>
<td>Figure 4.6</td>
<td>Optical micrograph of device</td>
<td>62</td>
</tr>
<tr>
<td>Figure 4.7</td>
<td>Signal conditioning circuit schematic</td>
<td>63</td>
</tr>
<tr>
<td>Figure 4.8</td>
<td>Wheatstone bridge control response</td>
<td>64</td>
</tr>
<tr>
<td>Figure 4.9</td>
<td>Melting response of wax</td>
<td>65</td>
</tr>
<tr>
<td>Figure 4.10</td>
<td>Photograph of bridge device integrated with PDMS microfluidics</td>
<td>67</td>
</tr>
</tbody>
</table>
Figure 5.1 Schematic: Typical AC wheatstone bridge ................................. 69
Figure 5.2 Lock-in amplifier: Working principle ........................................ 71
Figure 5.3 First order low-pass filter ....................................................... 72
Figure 5.4 System-level block diagram of experimental setup ....................... 74
Figure 5.5 Schematic representation of signal conditioning circuitry ............... 75
Figure 5.6 Photograph of acrylic jig for mounting PI flexible film device .......... 76
Figure 5.7 Process flow for microelectrode fabrication on polyimide flexible film . 77
Figure 5.8 Photograph of film device ...................................................... 79
Figure 5.9 Schematic: Droplet setup on device .......................................... 79
Figure 5.10 Optical melting curves of dsDNA ............................................. 80
Figure 5.11 Control and melting curves ................................................... 82
Figure 5.12 Control and melting curves ................................................... 83
Figure 5.13 Control and melting curves ................................................... 84
Figure 6.1 System-level block diagram of experimental setup ....................... 88
Figure 6.2 Schematic representation of custom electronic design .................. 88
Figure 6.3 Process flow: Polyimide film with copper equalizers .................... 89
Figure 6.4 Micrograph of the differential detection device ........................... 90
Figure 6.5 Schematic illustration of experimental setup for controlled heating ... 91
Figure 6.6 Constant power input .......................................................... 92
Figure 6.7 Fluorescence validation of DNA melting .................................... 94
Figure 6.8 Time response of the device .................................................. 95
Figure 6.9 Thermal response of device ................................................... 95
Figure 6.10 Melting characteristic of paraffin wax .................................... 95
Figure 6.11 Time response and RTD temperature mapping .......................... 96
Figure 6.12 Control and melting curves .................................................. 97
Figure 6.13 Experimental calculation of $Q$ ............................................. 98
Figure 7.1 Micrograph of differential detection device .................................. 101
Figure 7.2 Schematic: Cross-section of 3D double droplet system ................ 102
Figure 7.3 Photograph of 3D-droplet device ........................................... 103
Figure 7.4 Time response curves .......................................................... 105
Figure 7.5 Control and melting curves ................................................... 106
Figure 7.6 Time response curves .......................................................... 107
Figure 7.7 Control and melting curves ................................................... 108
Figure 7.8 Time response and device parameters ..................................... 109
Figure 7.9 RTD temperature mapping and control curve ............................. 110
Figure 7.10 DNA melting curves .......................................................... 111
Figure 7.11 Time response curves and RTD temperature mapping ................ 112
Figure 7.12 Control and boiling curves .................................................. 113
Figure 7.13 Photograph: Copper and copper-less devices ........................... 115
Figure A.1 3D glass-PDMS hybrid device ............................................. 146
Figure A.2 3D PI-PDMS hybrid device .................................................. 147
Figure A.3 Droplet based PI devices ...................................................... 147
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>thermal coefficient of resistance</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>thermal conductivity</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>thermal coefficient of resistance</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>thermal conductivity</td>
</tr>
<tr>
<td>$\rho$</td>
<td>resistivity, density</td>
</tr>
<tr>
<td>$\tau$</td>
<td>thermal time constant</td>
</tr>
<tr>
<td>$f_c$</td>
<td>corner or cut-off frequency</td>
</tr>
<tr>
<td>$Q$</td>
<td>amount of energy or heat transfer</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature</td>
</tr>
<tr>
<td>$R$</td>
<td>resistance</td>
</tr>
<tr>
<td>$I$</td>
<td>current</td>
</tr>
<tr>
<td>$V$</td>
<td>voltage</td>
</tr>
<tr>
<td>$A$</td>
<td>cross-sectional area</td>
</tr>
<tr>
<td>$P$</td>
<td>electrical power</td>
</tr>
<tr>
<td>$Z$</td>
<td>impedance</td>
</tr>
<tr>
<td>$T_m$</td>
<td>melting temperature</td>
</tr>
<tr>
<td>$C_p$</td>
<td>specific heat capacity</td>
</tr>
<tr>
<td>$Bi$</td>
<td>Biot number</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>$\Delta H$</td>
<td>enthalpy change</td>
</tr>
<tr>
<td>$\Delta G$</td>
<td>Gibbs free energy change</td>
</tr>
<tr>
<td>$\Delta S$</td>
<td>entropy change</td>
</tr>
<tr>
<td>$V_{pp}$</td>
<td>peak-peak alternating voltage</td>
</tr>
</tbody>
</table>
# ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI</td>
<td>deionized</td>
</tr>
<tr>
<td>PI</td>
<td>polyimide</td>
</tr>
<tr>
<td>TE</td>
<td>Tris-EDTA</td>
</tr>
<tr>
<td>IC</td>
<td>integrated circuit</td>
</tr>
<tr>
<td>TC</td>
<td>thermocouples</td>
</tr>
<tr>
<td>LP</td>
<td>low pass</td>
</tr>
<tr>
<td>A/D</td>
<td>analog to digital converter</td>
</tr>
<tr>
<td>MCA</td>
<td>melting curve analysis</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>LOC</td>
<td>lab-on-a-chip</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>DSC</td>
<td>differential scanning calorimetry</td>
</tr>
<tr>
<td>DTA</td>
<td>differential thermal analysis</td>
</tr>
<tr>
<td>ITC</td>
<td>isothermal titration calorimetry</td>
</tr>
<tr>
<td>RTD</td>
<td>resistance temperature detector</td>
</tr>
<tr>
<td>TCR</td>
<td>temperature coefficient of resistance</td>
</tr>
<tr>
<td>FEM</td>
<td>finite element method</td>
</tr>
<tr>
<td>DMM</td>
<td>digital multimeter</td>
</tr>
<tr>
<td>DIP</td>
<td>dual in-line package</td>
</tr>
<tr>
<td>DAQ</td>
<td>data acquisition</td>
</tr>
<tr>
<td>PLL</td>
<td>phase-locked loop</td>
</tr>
<tr>
<td>PFP</td>
<td>perfluoro pentane</td>
</tr>
<tr>
<td>IPA</td>
<td>isopropanol or isopropyl alcohol</td>
</tr>
<tr>
<td>USB</td>
<td>universal serial bus</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribose nucleic acid</td>
</tr>
<tr>
<td>dsDNA</td>
<td>double-stranded deoxyribose nucleic acid</td>
</tr>
<tr>
<td>ssDNA</td>
<td>single-stranded deoxyribose nucleic acid</td>
</tr>
<tr>
<td>PDMS</td>
<td>polydimethylsiloxane</td>
</tr>
<tr>
<td>PTFE</td>
<td>polytetrafluoroethylene</td>
</tr>
<tr>
<td>GPIB</td>
<td>general purpose interface bus</td>
</tr>
<tr>
<td>MODEM</td>
<td>modulator demodulator</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Recent discoveries in molecular biophysics have revolutionized applied fields such as forensic science, biotechnology and clinical diagnostics. Important findings related to the structural details of nucleic acids have led to key breakthroughs in sequencing the human genome, constructing clinical research and medical therapies. In particular DNA (deoxyribose nucleic acid) sequencing plays a key role in understanding the structural make-up of an organisms gene expression. Both optical and calorimetric methods are progressively moving toward understanding characteristic behavior of DNA via drug-DNA or protein-DNA interactions. Simultaneously efforts are undertaken to miniaturize these techniques in order to create portable diagnostics for enhancing point-of-care medical therapies.

In this thesis we discuss design concepts and novel fabrication methodologies for chip calorimeters to study the thermodynamic signatures of biological liquid samples. In addition, we present strategies to circumvent common drawbacks in fluid handling techniques such as evaporation and bubble-formation. We present four generations of chip calorimeters, giving a detailed account of each generation’s design, novel features, its functional drawbacks and the corresponding strategies for optimizing the subsequent design.

1.1 Purpose and Significance

Breakthroughs in the biomedical industry and silicon microfabrication techniques have inspired the creation of miniaturized sensor platforms capable of performing a broad spectrum of functions [143]. Miniaturized biosensors have huge implications in field testing for pathogen detection, environmental monitoring, patient bed-side diagnostics and food safety control. The current trend in the biomedical industry is to enhance portability by scaling down the size of table-top laboratory equipment for creating ‘lab-on-chip’ clinical diagnostics and point-of-care testing devices [227, 200]. Compact integration, easy portability, minimized consumption
of reagents and improved cost-effectiveness through reduced manufacturing and material costs have enabled the development of disposable biochips. Biochips in particular are designed for a wide variety of applications such as DNA analysis, immunoassays, drug toxicity monitoring and forensic analysis.

Processes such as polymerase chain reaction (PCR) and melting curve analysis (MCA) are the most common DNA analysis techniques used for a wide variety of applications in research laboratories and diagnostic tools. Conventional PCR and MCA table-top instruments employ bulky and expensive optical equipment making miniaturization immensely difficult. Although research groups have successfully shrunk MCA devices using varying degrees of micromachining most rely on probe-based fluorescence MCA which still entails the use of optics for the purpose of detection. The inclusion of such bulky and expensive optics makes these biosensors highly unlikely to be used in field testing or as a patient bed-side diagnostic tool.

Many research groups have devised microfabricated calorimeters for applications ranging from traditional polymer thermal studies to gas sensing. Only a few teams have demonstrated the ability of applying liquid samples to their microcalorimeters [284, 271] through heavy micromachining [278, 153]. However their techniques are not geared toward testing biomolecules suspended in solution.

In this thesis, we present a novel amalgamation of silicon microfabrication technology with custom microelectronic tooling for creating miniaturized microcalorimeters to harvest the thermal signature of DNA through MCA. We integrate liquid handling components and microsensors on the same platform and couple them with capture electronics. Compared to current chip calorimeters, our design features several distinguishing characteristics: easy fabrication using off-the-shelf raw materials, low sample volume, repeatability, versatility (capable of identifying thermal signatures of several kinds of samples) and portability. We also describe a novel technique to eliminate bubble-formation and control evaporation during a typical temperature scan.

1.2 DNA

1.2.1 Background

Watson-Crick hydrogen bonding of complementary single strands resulting in duplexes are the most common forms of nucleic acids [221]. Double-stranded deoxyribose nucleic acid (dsDNA) is one such nucleic acid molecule that exists as a compact 3-dimensional folded structure whose structural stability is dependent on intermolecular interactions [127]. The two strands forming the backbone of the ‘twisted ladder’ or the double helical structure are composed of sugar-phosphate molecules. These strands are held together by nitrogenous base-pairs. A nucleotide
is a subunit made of a base, a sugar and a phosphate. The primary structure of the double-helical DNA is defined as a set of nitrogenous bases stacked in a specified sequence [50, 82]. The types of bases for DNA are Adenine (A), Cytosine (C), Guanine (G) and Thymine (T) [70] where the Watson-Crick pairs are the complementary bases G-C and A-T [156]. The strands containing these base pairs are directional. For practical purposes the beginning of the strand is denoted as 5’ while the end is denoted as 3’. Complete base-pairing occurs when the reverse complement of one strand binds to it in an anti-parallel fashion. For instance a short base-pair sequence 5’ - ATGCT - 3’ will completely bind with its complementary base-pairs 5’ - AGCAT - 3’. Every base pair is bound together by hydrogen bonds. While the A-T bases are bound by two hydrogen bonds the G-C bases are bound by three. In other words DNA helices with higher G-C content have stronger interacting strands than those with higher A-T content [40].

1.2.2 DNA thermodynamics

The study of temperature effects on the structure of DNA is termed DNA thermodynamics. Automated PCR instruments loaded with dsDNA samples supply temperatures in the range of > 95 – 100°C for a few minutes to break hydrogen bonds between the base pairs. Even though the hydrogen bonds may break at lower temperatures the DNA mixtures are heated to near boiling in order to ensure that all the DNA has denatured to ssDNA which is imperative for high throughput in the consecutive steps. Upon cooling to 65°C the base pairs readily reform [139]. A thermodynamic driving force is responsible for base pair stacking and is enthalpic in nature [203, 7]. Hunter et al. [119, 120] have explained that this stacking energy is acquired by electrostatic interactions and Van der Waals’ forces with the neighboring nucleotides. Such neighbored-nucleotide interactions are energetically favorable [146] and interactions between the base pairs is vital to the structural stability of a DNA duplex in solution [86, 58, 288]. In other words the nucleotide bonds that have formed help the neighboring nucleotides to pair as well. A homogenous solution of dsDNA is often subjected to a temperature scan to break these bonds in order to study the kinetics of the reaction. Such an event is termed as ‘melting’. The melting temperature (Tm) defines how easily a dsDNA is formed and whose stability depends on the nucleotides that form the bonds.

**Theoretical determination of Tm**

There are several methods to determine the Tm value theoretically. The simplest approximation is the ‘2+4 rule of thumb’ or the ‘Wallace rule’ method that simply assigns 2°C to each A-T pair and 4°C to each G-C pair [281] suspended in 0.9 M NaCl solution. The sum of all the values of the individual pairs in dsDNA is the final Tm value. Though the Tm value is easy to compute the ‘2+4 rule of thumb’ suffers drawbacks. First the resulting Tm maybe very inaccurate as it
does not allow adjustments in the salt concentrations. Second the rule may be applied only to dsDNA sequences that are 20 – 40 nucleotides long.

A more advanced technique is the ‘Linear regression’ which is based on both the G-C ratio and the length of the DNA molecule \([289, 13, 25]\). The G-C ratio is obtained by calculating the ratio of the number of G and C nucleotides along with prior knowledge about the total length of the DNA. Various research teams have included a molar sodium concentration correction factor in the equation to account for the interactions of the DNA in solution. The ‘Linear regression’ method is more accurate than the ‘2+4 rule of thumb’ rule. However it does not account for the stacking effects of the nucleotides which in turn affects the overall enthalpy of the system.

Nobel laureate Arthur Kornberg \([147, 131]\) devised the ‘nearest-neighbor’ method to determine the melting characteristics of DNA. The method accounts for enthalpy of base pair formation and the stacking effect between the nearest-neighbor nucleotide pairs \([238]\). The nearest neighbor method assigns an enthalpy and entropy value to each base pair along with its interaction with the previous pair in a pre-calculated table to obtain the sum of all the values. Some of the widely accepted thermodynamic tables are found calorimetrically \([30, 242]\) using carefully designed oligonucleotide probes. Breslauer et al. \([30]\) used differential scanning calorimetric measurements to tabulate enthalpy change values for pair-wise interactions as seen in Table 1.1. Entropy change \(\Delta S\) and free-energy change \(\Delta G\) were calculated at 25°C using similar calorimetric principles. With knowledge of the base pair sequence and their relative enthalpies the \(T_m\) value can be predicted using the equilibrium equation (1.1a). An illustration of the theoretical calculation of \(\Delta H\) value for a high G-C content DNA sequence has been shown in Figure 1.1 using the equation (1.1b) and enthalpy change values from Table 1.1. The experimentally determined enthalpy value for the example DNA sequence \(\Delta H_{\text{experimental}}\) was found to be 59.6 kcal.

\[
\Delta G = \Delta H - T\Delta S \quad (1.1a)
\]

\[
\Delta H_{\text{total}} = \sum \Delta h \quad (1.1b)
\]

In equation (1.1a) \(\Delta G\) is the free energy change, \(\Delta H\) is the enthalpy change, \(T\) is the temperature in kelvin and \(\Delta S\) is the entropy change associated with the thermal denaturation of the DNA. in equation (1.1b) the \(\Delta H_{\text{total}}\) is the total enthalpy change obtained by summing the individual \(\Delta h\) values of interactions between base pairs.

The overall \(\Delta G\) can be calculated by employing the \(\Delta G\) and \(\Delta S\) values tabulated \([30]\) (not shown) to understand the energy equilibrium of the system.
Table 1.1: Nearest-Neighbor Nucleotide Enthalpy Chart

<table>
<thead>
<tr>
<th>Nucleotide base pair interactions</th>
<th>$\Delta h$ (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA/TT</td>
<td>9.1</td>
</tr>
<tr>
<td>TA/AT</td>
<td>6.0</td>
</tr>
<tr>
<td>GT/CA</td>
<td>6.5</td>
</tr>
<tr>
<td>CG/GC</td>
<td>11.9</td>
</tr>
<tr>
<td>GC/CC</td>
<td>11.2</td>
</tr>
<tr>
<td>GG/CC</td>
<td>11.0</td>
</tr>
<tr>
<td>GC/CG</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Figure 1.1: Method for predicting total transition enthalpy for DNA oligomer: The example DNA sequence was tested calorimetrically for validation and the experimental $\Delta H_{\text{experimental}}$ was determined to be 59.6 kcal [30].

1.3 Melting Curve Analysis

Generating melting profiles of biomolecules is a powerful tool in molecular biology [125]. Thermal denaturation or ‘melting’ is a fundamental nucleic acid property that allows for the assessment of specific nucleotides forming a DNA sequence. Typically, the sample is heated through a range of temperatures while its optical properties such as ultraviolet (UV) absorbance or fluorescence are recorded.

1.3.1 UV absorbance melting curves

Historically, the melting of double-stranded DNA (dsDNA) was monitored by UV absorption measurements from a quartz cuvette placed inside a UV spectrophotometer. A steep increase of 35—40% in optical density or UV absorbance at 260 nm [19, 163] is observed when a DNA duplex
structure denatured into its single-stranded DNA (ssDNA) constituents [140]. Such an effect is termed ‘Hyperchromicity’ [171]. The stacked configuration of base pairs within the double helix results in a II−II interaction between them which diminishes the extinction coefficient. However when the DNA solution is supplied with enough thermal energy to disarray the stacked base pairs and melt the dsDNA into its ssDNA components the free individual nucleotides that are exposed to the solvent absorb more UV than when they were stacked. The melting temperature at which half of the hyperchromicity of the DNA has occurred depends on the DNA strand length, base composition and the ionic strength of the suspension solution [27]. Although the UV absorbance technique is simple it is seldom used due to a number of drawbacks. The technique requires several µgs of DNA in solution [290], takes several hours to complete due to the slow heating rates 0.1−1°C/min [3], has low throughput owing to the sample holder limitation of the spectrophotometer and the loss of sensitivity when resolving melting curves of triplexes and quadruplexes [62].

1.3.2 Fluorescence melting curves

Fluorescent DNA melting analysis is more popular than UV absorbance due to its higher sensitivity and minimal sample volume [293] requirement. Fluorescent melting curve analysis (MCA) was first reported by Witter et al. [235] in the late nineties who demonstrated its use as an integral part of real-time PCR. It was introduced as a tool meant for performing post-PCR analysis to increase the specificity [28]. PCR products at the end of every amplification cycle were analyzed to confirm if the appropriate target amplification had occurred. This method reduced the risk of carrying over a contaminant to the successive amplification cycles. MCA was quick to gain popularity because of its simplicity and remarkable speed. The method enabled subsequent interrogation with the samples due to its non-destructive nature [71].

Melting curve analysis is performed by mixing double-stranded DNA (dsDNA) with an intercalating dye and heating the mixture while measuring its fluorescence intensity. The dsDNA melts into single-stranded DNA (ssDNA) as it is heated above its melting temperature. The dye is quenched by ssDNA, resulting in a decrease in fluorescence intensity during heating as seen in Figure 1.2. This method is used for proof of concept in section 3.3.2. The shape of the resulting melting curve provides information about the dsDNA length, base pair content, and single nucleotide polymorphisms [235]. For example, longer dsDNA strands tend to melt at higher temperatures; single nucleotide polymorphisms tend to reduce the melting temperature [22] during a fluorescence resonance energy transfer (FRET) event.
1.3.3 Review: Current research

Many research groups have attempted to miniaturize table-top MCA to a portable lab-on-chip platform because MCA would make an attractive portable assay. It could be used to diagnose genetic diseases at the point-of-care. Commercial MCA systems are bulky and require sample volumes of about 150 µL and sample concentrations of 0.2 mg/ml. Analysis times are on the order of 15 - 90 min. Miniaturization offers several ways to improve MCA: rapid heating times (1000 °C/s), reduced sample volume (≤ 1 µL), and reduced temperature variation within the sample volume, yielding a higher signal to noise ratio. Microscale MCA has been performed with devices that use mechanical [23], surface plasmon resonance [88], chemical [237, 207], and optical detection [259, 72] schemes. Optical detection techniques are attractive because they typically do not require tethering oligos to a substrate, thus reducing a surface functionalization step.

In addition to an optical system, a heater and thermometer are required to measure the precise dsDNA melting temperature. Sundberg et al. performed single nucleotide polymorphism (SNP) genotyping on a MCA device bonded to Peltier elements [259]. Dodge et al. designed a

![Fluorescence melting curve analysis](image)

Figure 1.2: Fluorescence melting curve analysis: A sharp decrease in the dye fluorescence is observed when ssDNA is formed at the end of the heating cycle. The melting temperature $T_m$ is the temperature at which the sample solution contains equal quantities of dsDNA and ssDNA.
microfluidic platform in which molecular beacons were used a temperature probes inside the reaction vessel to study dsDNA melting characteristics [72]. Gale et al demonstrated a MCA device, using off-chip heating and sensing elements[57].

1.4 Calorimetry

1.4.1 Calorimetry as a sensing tool

The word calorimetry is derived from Latin: ‘calor’ meaning ‘heat’ and ‘metrium’ meaning ‘to measure’. Calorimetry is the science of heat which involves thermal analysis techniques to measure heat flow transitions in materials as a function of temperature and time [162]. Calorimetric measurements yield important information about the thermodynamic signature of the sample involving endothermic or exothermic processes [276, 93]. Ideal calorimeters fitted with temperature sensors within large sample holders offer high sensitivity measurements due to the large ratio of heat producing volume to the overall outer surface area available for heat exchange [280]. The advantages of using calorimeters as sensors are multi-fold. First, heat transformations occur naturally in almost all biological and chemical processes [182, 199]. Second, harnessing this heat as a universal measurement parameter is key to creating label-free sensors, thus avoiding unnecessary sample modifications. Third, the non-invasive nature of sensing allows for further investigation of the samples without manipulation. Fourth, the physical and structural properties of the samples such as opacity or porosity do not hinder calorimetric measurements [85]. Lastly, the temperature sensors in a calorimeter may be stationed in an area that is protected from biofouling, thereby increasing the life-time and accuracy of the calorimeter.

1.4.2 Evolution of chip calorimetry

For over a century calorimeters have been optimized to profile thermal characteristics with the highest possible sensitivity and resolution [37]. Their applications however have been strictly confined to laboratory usage because of their bulky nature. Conventional calorimeters yield high quality results only with large volumes of the sample and slow heating rates [4]. Measuring the thermal patterns of trace amounts of biomolecules or chemical reactions yields low signal to noise ratios because the heat flow into the sample is reduced [292, 182]. In addition, the large thermal time constants owing to the bulky heat sinks associated with conventional calorimeters make them immobile and slow [154]. Miniaturization of the conventional table-top calorimeters [181] allows for the creation of hand-held, low-cost sensor devices that can have a wide spectrum of field applications [12].
1.4.3 Current research: Chip calorimetry

Rapidly developing fields of silicon microfabrication and polymer microfluidics have aided research teams to miniaturize calorimeters which ultimately led to the creation of chip calorimeters [224]. Chip calorimeters have since demonstrated potential for use as gas sensors [38, 106] and for thermal characterization of thin films [90, 303], biomolecules [15], polymers [64] and bioorganisms [299, 29]. Examples of contemporary calorimeters along with their design features and relevant applications have been highlighted below.

Micromachined chip calorimeters

Many groups have used intensive microelectromechanical (MEMS) and other CMOS (complementary metal oxide semiconductor) foundry techniques for developing thin dielectric membranes as sample holders. Fominaya et al. [90] designed a nanocalorimeter for measuring specific heats of thin films as seen in Figure 1.3(a). The leads from the serpentine heater patterned on the sample holder seen in the image were passed on twelve bridges to connect to the main silicon frame for interfacing with real-world electronics. Heavy-duty calorimetric chips [277] as seen in Figure 1.3(b) were designed by gluing microfabricated silicon chips in a ceramic pin-grid array (PGA) for measuring the heat of reaction of enzymatic coatings. Similarly Von Arx et al. [210] micromachined bulk silicon for creating cantilever beams to support electrical connections as seen in Figure 1.3(c) for chemical sensing.

![Micromachined chip calorimeters: (a) Nanocalorimeter for measuring specific heat of thin films [90] (b) Silicon chip glued to a ceramic main frame for measuring heat of reaction of enzyme monolayers [277] (c) Chemical sensing using micromachined bulk silicon cantilever beams [210].](image)

Figure 1.3: Micromachined chip calorimeters: (a) Nanocalorimeter for measuring specific heat of thin films [90] (b) Silicon chip glued to a ceramic main frame for measuring heat of reaction of enzyme monolayers [277] (c) Chemical sensing using micromachined bulk silicon cantilever beams [210].
Membrane based chip calorimeters

Another prominent approach for creating chip calorimeters was to incorporate thin membranes as a crucial component of the designs. As seen in Figure 1.4(d) Torres et al. report a polyimide membrane based nanocalorimeter capable of measuring the heat produced from binding reactions of enzymes in open air [271]. Although the microelectrodes on the membrane were employed for droplet manipulation they were not designed for temperature scanning. Schick et al. studied the recrystallization kinetics in polyethylene terephthalate (PET) while heating a dry amorphous sample as seen in Figure 1.4(e) on a silicon nitride membrane [193]. Roukes et al. developed a silicon chip with metallized electrodes integrated with PDMS and parylene microfluidics for measuring heat of reaction for biochemical reactions [161]. The stand-alone calorimetric unit was capable of measuring enthalpy reactions in liquid samples. However it was necessary to implement a vacuum environment using bulky addenda and external vacuum facilities in order to enhance the sensing capabilities as seen in Figure 1.4(f).

![Figure 1.4](image1.png)  
**Figure 1.4**: Chip calorimeters with membrane components: (d) Polyimide membrane based chip calorimeter for measuring enthalpy of reactions in droplet samples [271] (e) Thin silicon nitride membrane based chip calorimeter for measuring enthalpy of phase transition in dry samples [193] (f) Silicon integrated with polymer membrane microfluidics for measuring enthalpy changes in liquid samples [161].

1.4.4 Advantages of current design

Our design aims at eliminating optics to perform on-chip MCA. As seen in section 1.3.3 optical MCA not only increases the manufacturing costs but also reduces the portability of the system due to the bulk added by the optics. In addition label-free detection would be impossible which
invariably increases sample preparation time.

The overview of current chip calorimetry research presented above suggests that most chip calorimeters are developed for testing dry samples exposed to ambient air. Such open-air techniques present several drawbacks when testing liquid samples: evaporation and associated changes in biomolecule concentration and sample containment. The micromachined chip calorimeters have several drawbacks. These fabrication-intensive chip calorimeters have delicate sample holders that are not conducive for testing liquid samples. Human error during sample loading maybe introduced in such devices as they present challenges for manual handling and manipulation. In addition the fragility of the sample holders restricts easy restoration and reusability. Similarly the membrane-based designs do not support detection of biomolecules suspended in liquid medium. Calorimetrically determining enthalpy changes during phase transitions of biomolecules suspended in a liquid medium has not been developed yet. Although the soft polymer integrated microfluidics by Roukes et al. [161] demonstrates the feasibility of testing liquid samples the polydimethylsiloxane (PDMS) reaction chamber suffers drawbacks such as permeability to gases resulting in evaporation and the addition of thermal mass which could result in decreasing the sensitivity of the device.

Our non-optical MCA device design aims at reducing overall thermal mass of the chip calorimeter along with increasing thermal isolation without using peripheral addenda. The substrate materials used in the design not only provide strong mechanical support but are also cost-effective and easily available. Consequently the robust substrates allow for easy sample manipulation or loading thus reducing variability due to human error. Both solid and liquid samples can be tested on our devices with emphasis on enthalpy reactions involving biomolecules suspended in liquid medium. Batch processing or mass production of our device was made possible as the fabrication principles followed traditional silicon microfabrication methodologies without the requirement for heavy micromachining or a controlled cleanroom environment.

1.4.5 Applications in life sciences

Historically, calorimetric tools were not widely used for life science applications primarily due to two reasons. First, because the sample volume required is in the range of a milliliter to a fraction of a milliliter, which limits studies that can be performed on trace macromolecules or drug samples. Second, conventional calorimeters suffer from low resolution when measuring thermodynamic properties of highly diluted biological samples. However advances in calorimetric techniques have opened up ways which may be used in the measurement of thermodynamic protein and nucleic acid folding and binding interaction data [55, 101]. By integrating fluid handling capabilities with chip calorimeters, researchers have expanded their use as biomolecular recognition tools [161]. Chip calorimeters have enabled the thermodynamic study of biomolecules and
have helped in revealing the fundamental concepts of molecular organization, cellular function and the key energetics that maintain the stability and integrity of a 3-dimensional biomolecule [206, 183]. Chip calorimeters are a unique combination of heating and sensing elements, sample handling units and capture electronics - all integrated on a single platform. Thus chip-based microcalorimetry enables portable, label-free and high-throughput sensing with minimal sample volume consumption. This project involves the fabrication and characterization of a chip calorimeter capable of detecting DNA melting by applying principles of differential thermal analysis. The use of cheap off-the-shelf raw materials and silicon batch processing to manufacture our chips establishes an easy route to creating cost-effective, portable and disposable biosensors with rapid response times.

1.5 Types of Thermal Analysis Techniques

Thermal analysis describes the process of monitoring data and subsequent interpretation to enable thermal profiling of a material. Most thermal analysis techniques measure a physical property of the material under test as a function of temperature while the material is subjected to a controlled temperature program [176]. Heat flow into a material induces physical and chemical transformations which provide useful information for the identification and characterization of that material [176]. Table 1.2 lists the most common thermal analysis techniques used today and the corresponding property of the material they measure [59, 31, 295, 78]. In the following sections we describe the purely thermal techniques DSC and DTA. They are closely related since DSC measures the heat flow difference between a sample and reference which can be observed when a temperature difference arises in the sample [81]. DSC allows for direct measurement of thermodynamic processes such as enthalpy or phase transition [60]. DTA on the other hand measures the temperature differences between the sample and reference but has

<table>
<thead>
<tr>
<th>Technique</th>
<th>Common name</th>
<th>Measured material property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential Scanning Calorimetry</td>
<td>DSC</td>
<td>Heat flow difference</td>
</tr>
<tr>
<td>Differential Thermal Analysis</td>
<td>DTA</td>
<td>Temperature difference</td>
</tr>
<tr>
<td>Thermomechanical Analysis</td>
<td>TMA</td>
<td>Dimension</td>
</tr>
<tr>
<td>Thermogravimetric Analysis</td>
<td>TGA</td>
<td>Mass</td>
</tr>
<tr>
<td>Dynamic Mechanical Analysis</td>
<td>DMA</td>
<td>Modulus</td>
</tr>
</tbody>
</table>

Table 1.2: Common Thermal Analysis Techniques
to be calibrated to perform enthalpy measurements. This thesis involves the development of an elegant DTA device capable of detecting enthalpy changes in the range of 2 µJ from sub-microliter sample volumes. Most chip calorimeters as seen in section 1.4.3 employ the method of DSC for measuring changes in the differential power as a means of determining the enthalpy changes in the sample. Our method involves measuring the temperature changes directly occurring within the sample. A computer controlled DC power unit was customized to supply programmed power to the heater for delivering a linear temperature scan on the sample. Such a ramp-up constant power technique minimized output non-linearities in the DTA chip calorimeter setup.

1.6 Differential Thermal Analysis

Le Chatelier et al. [43] first employed the method of uniform heating and cooling of a system to study the phase transformations through temperature-time recordings [63].

1.6.1 Principle of operation

The experimental setup as seen in Figure 1.5 consists of subjecting containers holding a sample and an inert reference to uniform heating or cooling while recording the temperature difference between them as a function of time [279]. A temperature difference is noted when the sample undergoes a phase change involving release or absorption of heat. Once the sample completes its phase transition the temperature difference reduces and eventually follows baseline. Hence, a temperature ramp up from below the equilibrium temperature to above it will allow the sample to undergo the complete transformation. Figure 1.6(a) shows typical DTA scans. The input temperature scan shown in the inset is useful to examine thermodynamic properties that occur close to the equilibrium temperature. When both sample and reference containers are heated at the same temperature rate scan (as seen in the inset) their temperatures rise steadily as the reference material will not undergo physical or chemical transitions. However when the sample melts its temperature will lag behind the reference curve as it absorbs heat energy required for melting. There is no temperature change within the sample during this ‘lag’ period. When the sample completes its phase transition it follows the reference curve for the rest of the scan. Figure 1.6(b) shows the curve resulting from the difference of the sample and reference curves. The area \( A \) within the endothermic curve is proportional to the enthalpy \( \Delta H \) of the reaction and is governed by the equation (1.2)

\[
\Delta H = K A = K \int \Delta H dt
\]
Figure 1.5: DTA operation principle: The block diagram shows a typical DTA experimental apparatus. The electrically generated heat is identical in both heaters to supply the equal amounts of energy to both containers.

Figure 1.6: DTA thermogram: (a) Differential heating curves: The inset shows the input temperature scan profile. The sample curve lags behind the reference curve as it absorbs heat necessary for melting. (b) Resultant of differential curves: graph of difference between sample and reference curves from (a). The downward peak shows a heat-absorbing or endothermic event.

where the constant $K$ depends on the thermal properties of the sample which vary with temperature.
1.6.2 Applications

The extensive development of instrumentation has expanded the scope of DTA for investigating organic plant matter [195]. With small quantities of the sample Collin et al. [41] demonstrated that the melting temperature of natural and artificial phospholipids are dependent on the saturation levels of the component hydrocarbon chains. The thermal behavior of aqueous dispersions of various fatty acids, proteins and plasma membranes were studied using DTA [186].

1.7 Differential Scanning Calorimetry

This technique was first introduced in 1960 by O’Neill et al. [124]. Differential Scanning Calorimetry (DSC) is a method designed to study thermally induced phase transitions. DSC measures the excess heat capacity of the system when the sample undergoes a conformational change as in biological membranes, proteins [272, 94, 240] or double-stranded DNA [32].

1.7.1 Principle of operation

In general DSC is a technique for measuring the input energy required to maintain near-zero temperature difference between a sample and reference material while they are subjected to identical temperature programs. A schematic of a general DSC experiment is as seen in Figure 1.7(a). The sample and reference cells are enclosed within the same furnace. $P_R$ and $P_S$ are input energies which are simultaneously introduced to the two cells. The thermal sensors $T_R$ and $T_S$ provide dynamic feedback on the internal temperature of the cells while the heaters raise the temperatures of each cell identically over time. The recorded difference in the input energy which is required to match the temperature of the sample to that of the reference is the amount of energy that the sample is absorbing or emitting in excess. The rate of change of input energy (heat flow) to each cell against average temperature is recorded. Finally the difference plot of the reference subtracted from the sample yields the specific heat capacity of the sample ($C_p$) with respect to time. An example of such a DSC thermogram is shown in Figure 1.7(b). The peak of the DSC curve is indicative of the melting temperature ($T_m$) of the sample. The area under the curve can be used to calculate the enthalpy ($\Delta H$) of the system.

1.7.2 DSC capabilities

Nucleic acids such as DNA and several forms of proteins undergo structural changes when subjected to heat [258]. Such biological systems experience complex thermodynamic interactions while in solution. The net effect of these interactions can be easily interpreted by DSC. DSC is one of the few methods capable of directly measuring the heat of the system or enthalpy ($\Delta H$)[127, 295, 104]. It can be applied for thermal analysis of materials that undergo phase
Figure 1.7: (a) Schematic illustration of typical DSC experimental setup. The sample and reference cell are maintained at isothermal conditions. (b) Typical DSC thermogram: The specific heat curve ($C_p$) of the sample is obtained from the difference of the reference and sample heat flow curves. The peak integral denotes the enthalpy ($\Delta H$) of the system.

transitions and conformational changes. These physical transformations involve temperature changes and can be tracked through the material’s melting, glass transition and crystallisation [112, 54]. DSC monitors heat absorbed or radiated by the sample relative to that of a reference material as a function of time [108, 239]. DSC has been used as an effective bedside diagnostic tool [99] for early diagnosis and screening of cancer patients [98, 87]. Several autoimmune diseases which exhibit plasma proteome characteristic can be captured by DSC to produce a disease signature thus making it an invaluable tool in disease monitoring [39].

### 1.8 Heat Transfer

The transfer of heat is generally from a region of high temperature to a region of low temperature and follows the law of conservation of energy or the first law of thermodynamics as seen in equation (1.3)

$$\Delta U = Q - W$$  \hspace{1cm} (1.3)

where $\Delta U$ is the internal energy change in the object, $Q$ is the energy added to the system and $W$ is the work done by the system. In other words the law states that energy is always conserved and cannot be created or destroyed but merely transformed from one form to another. Heat
transfer occurs either through conduction, convection or radiation.

1.8.1 Conduction

Conduction is a means of heat transfer within the body of a material through molecular agitation but without the motion of the body as a whole. For example if one end of a metal rod is heated the heat energy will be transferred to the cooler side of the rod by the bombardment of the hot high speed particles with the slow particles causing a net transfer of heat to the cooler particles. Conduction can be calculated using the equation (1.4)

\[
\frac{Q}{t} = \frac{\kappa A (T_{\text{hot}} - T_{\text{cold}})}{d}
\]

(1.4)

where \(Q\) is the heat transferred in time \(t\), \(\kappa\) is the thermal conductivity of the material in \(W/mK\), \(A\) is the cross-sectional area of the material and \(T\) is the temperature.

1.8.2 Convection

Convection is a means of heat transfer by the mass motion of fluids such as water or air. When fluids are heated they carry the heat energy with them even while away from the heat source. For example air above a hot surface such as a stove gets heated since hot air expands and becomes less dense causing it to rise. Similarly hot water is less dense than cold water. Assume that water is being heated in a metal vessel. As hot water becomes less dense its buoyancy increases causing it to rise while simultaneously pushing the cold water downward - all in the presence of gravity. Such a motion where hot water rises and cold water descends leads to convective circulation within a liquid without an external driving force. These induced flows are opposed by viscous drag at the interface of the liquid and solid. The Grashof number compares such interrelated buoyancy and viscous forces. The Grashof number is the ratio of the buoyant and viscous forces of a fluid [135, 122]. Convective currents within a fluid have been explained further in section 2.5.1.

1.8.3 Transient heat transfer analysis

Thermal analysis of an object depends on its resistance to heat transfer both external and internal to its material. For example when a solid object is dropped in a hot water bath the object experiences external resistance to heat transfer. The resistance to heat transfer is first encountered at the interfacial surface between the solid and liquid followed by the internal resistance within the body of the object to the flow of heat from its outer surface toward the
center. Such a relationship between the internal and external resistances of an object can be defined by a quantity termed Biot number $Bi$.

### 1.8.4 Biot number ($Bi$)

Biot number is a dimensionless system parameter defined qualitatively as the ratio of internal to external resistances as seen in equation (1.5).

$$Bi = \frac{\text{Internal conductive resistance within the object}}{\text{External convective resistance at the surface of the object}}$$  \hspace{1cm} (1.5)

The physical quantity that $Bi$ describes determines the type of thermal system involved in the heat transfer. The Biot number maybe defined quantitatively as in the equation (1.6) below

$$Bi = \left( \frac{L_c}{\kappa} \right) \left( \frac{1}{h} \right) = \frac{h L_c}{\kappa}$$  \hspace{1cm} (1.6)

where $L_c$ is the length of the body defined as ratio of the volume of the body and its cross-sectional area, $h$ is the convective heat transfer coefficient and $\kappa$ is the thermal conductivity of the body in W/mK. Equation (1.6) elucidates that both the numerator and denominator in equation represent thermal resistance components of the system as previously described in equation (1.5). From equation (1.6) it is clear that $Bi$ will be much less than 0.1 when the internal thermal resistance is very low. In simpler terms, when $Bi$ is much less than unity, then most of the temperature drop is in the surrounding fluid while the solid may be considered isothermal. Consequently if the Biot number is very low the thermal system can be analyzed using a lumped capacitance model.

### 1.8.5 Lumped analysis

A lumped system is characterized by modeling the thermal system into discrete lumps. It is one in which the dependence of temperature on position (spatial dependence) is disregarded. In other words the temperature can be modeled as a function of time and the temperature differences inside each lump is negligible. Such a lumped model is acceptable since the Biot number ($Bi$) is much less than unity [122]. In this thesis all devices compose nickel thin-film microelectrodes which have low thermal resistance and hence heat transfer between the solid heater and the fluid sample were analyzed as lumped capacitance models.

During conventional operation in air heat losses from the system to the environment by means of thermal radiation [160] and convection [244] were ignored as the relative size of the heaters was small and the corresponding temperature ranges were not very high. In addition as
the Grashof number for microelectrode heater environments was low [159] it was assumed that heat losses through free convection in air was negligible. Therefore heat transfer was modeled as occurring primarily by conduction through air and the membrane. Microcalorimetric techniques involving thermal efficiency measurements in air and vacuum have revealed that heat loss by conduction in air is \( \approx 30\% \) greater than that through the membrane [287, 248].

1.8.6 Estimation of thermal capacitance of device

At constant pressure the heat capacitance of a system is defined as the energy flow into and out of the system to maintain energy balance. In this thesis the total heat loss of the device was estimated as the sum of heat losses to air and through the substrate. Heat losses are dependent on the thermal resistance of the materials involved in heat transfer. The heat capacity \( (C_p) \) of a calorimetric system is the amount of heat required to raise the temperature of the system by 1 K. Hence it follows that a system with low heat capacity would have a higher ability to detect smaller enthalpy changes than that of a system with high thermal capacity. A schematic of the device cross-section is shown in Figure 1.8. Cylindrical analysis [148, 244, 287] was used to model the device. The analysis involved using circular geometries for both the membrane and heater regions with equivalent areas and represented in the cylindrical coordinate system. Heat loss was modeled as the combined effect of heat conductance out of the heater and the

![Figure 1.8: Heat transfer model: Lumped analysis (a)Cross-sectional view of the device components involved in heat transfer. \( r_o \) is the radius of the substrate considered for modeling heat loss to air via conduction (b)Top-view of device showing the lateral dimensions of the heater \( (L_{heater}) \) and substrate \( (L_{subs}) \) contributing to heat loss via the substrate.](image-url)
membrane. Heat loss via conductance through air $Q_{\text{air}}$ and membrane $Q_{\text{subs}}$ are defined by the energy balance equations (1.7a) and (1.7b) respectively

$$Q_{\text{air}} = \frac{4\pi \kappa_{\text{air}}}{(1/r_i) - (1/r_o)} \quad (1.7a)$$

$$Q_{\text{subs}} = \frac{2\pi \kappa_{\text{subs}} T_{\text{subs}}}{\ln(r_{\text{subs}}/r_{\text{heater}})} \quad (1.7b)$$

where $\kappa_{\text{air}}$ is the conductivity of air and $\kappa_{\text{subs}}$ is the conductivity of the substrate in W/mK. The set of equations (1.8) define the various radii involved in the analysis

$$r_{\text{heater}} = \sqrt{\frac{L_{\text{heater}}^2}{\pi}}, \quad r_{\text{subs}} = \sqrt{\frac{L_{\text{subs}}^2}{\pi}}, \quad r_o = \sqrt{\frac{3L_{\text{subs}}^2}{2\pi}}, \quad r_i = \sqrt{\frac{L_{\text{heater}}^2}{2\pi}} \quad (1.8)$$

For heat loss through the substrate of thickness $T_{\text{subs}}$, $r_{\text{heater}}$ was considered as the radius of the heater while $r_{\text{subs}}$ was the radius of the substrate. Similarly for heat loss through air $r_i$ was considered as the radius of the heater while $r_o$ was the radius of the substrate.

The heat capacity of the device dictates the sensitivity of the device. The detection resolution of the device is indirectly proportional to its thermal capacitance. The goal of this thesis is to design microcalorimeters with low thermal capacitance and high temperature detection resolution. An account of the strategies involving concurrent design improvements with electronic detection methodologies have been presented.
Chapter 2

Design Considerations

2.1 Thin Film Heater/Sensor Design

2.1.1 Evolution of thin film technology in microsystems

Heaters and temperature sensors are essential components of a PCR or MCA experimental setup. Peltier elements have been historically used as heaters [291, 265, 166] but are not suitable for integration within a microchip due to their bulky nature and high power consumption [83]. Thin film elements have many advantages over conventional Peltier elements. First, thin film elements can be easily incorporated into microsystems using fairly traditional silicon microfabrication techniques [246, 301, 97, 304] on a variety of substrate materials. These microfabrication techniques enable precision positioning of thin film microelectrodes [223, 113]. Second, they offer excellent thermal management solutions because they operate on relatively low power [298, 205]. Third, they have small time constants owing to their small size and hence respond rapidly to input stimulus [220, 61, 103]. Lastly, with good design parameters metallic thin films can be used simultaneously as heaters and temperature sensors on the same substrate [117, 300, 250].

Similar to the PCR microsystems summarized above, calorimeters designed on microchip platforms also require miniaturized heater and temperature sensor elements for performing localized heating and sensing. Microcalorimeters designed for biochemical reactions confine fluid samples in reservoirs that are closely coupled with thin film heaters and thermometers [258, 112, 302].

In this thesis thin film resistive nickel heaters and temperature sensors were patterned on a variety of substrates to fabricate microcalorimeters. The rationale behind the type of sensor, its design parameters and choice of material are as described in the following sections.
2.1.2 Types of thermal sensors

The three main temperature sensor technologies that allow miniaturization are thermocouples, thermistors and resistance temperature detectors (RTDs). All three types of sensors measure temperature by directly converting it to a readable electrical signal.

Thermocouple

Thermocouples (TCs) are temperature sensors based on the thermoelectric effect. A thermoelement circuit is formed when two different metal wires are connected together at a junction and a closed circuit is formed with a second junction by joining the other two wire ends. When one junction experiences a different temperature than the other a thermoelectric voltage proportional to this temperature difference is produced by the circuit. This thermoelectric voltage generates an electric current that flows through the circuit and is dependent on the type of metals used and the temperature difference between the two junctions [91]. For practical reasons the sensing junction is called ‘hot junction’ while the reference junction held at a constant temperature is called ‘cold junction’ [136]. If both junctions experience the same temperature the voltage produced at each junction will be the same and hence no current will flow. Although TCs offer fast response times, sense a wide temperature range, are self-powered and simple to fabricate they lack measurement linearity and accuracy [219].

Thermistor

Thermistors are negative temperature coefficient (NTC) sensors [92]. They are fabricated using semiconductor metal oxides whose resistance decreases with increasing temperature. They exhibit a large change in resistance per degree change in temperature [255]. However, due to the number of electrons that increase exponentially with rise in temperature they suffer from severe non-linearity limiting their useful temperature sensing range to less than 100°C. They also suffer from baseline drift when measuring alternating temperatures [267].

RTD

Resistance temperature detectors (RTDs) are metal wires whose resistance increases with increasing temperature at a predetermined rate. Although slower to respond than TCs RTDs exhibit the most linear response when compared to both TCs and thermistors [188]. Also the voltage drop produced during sensing by the RTDs is much larger when compared to the other two. Along with long-term stability, RTDs also present easy fabrication solutions [174]. For the reasons stated above RTDs are chosen as the temperature sensors over thermistors and TCs.
A summary of general characteristics [152, 123, 174, 267, 257] of these sensors is as seen in the Table 2.1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Thermocouples</th>
<th>Thermistors</th>
<th>RTDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>Fair</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Fair</td>
<td>Fair</td>
<td>High</td>
</tr>
<tr>
<td>Repeatability</td>
<td>Fair</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Cost</td>
<td>Cheaper</td>
<td>Cheap</td>
<td>Cheapest</td>
</tr>
<tr>
<td>Ease of fabrication</td>
<td>Fair</td>
<td>Fair</td>
<td>High</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Low</td>
<td>High</td>
<td>Fair</td>
</tr>
<tr>
<td>Response time</td>
<td>Fast</td>
<td>Fast</td>
<td>Fair</td>
</tr>
<tr>
<td>Temperature range</td>
<td>Wide</td>
<td>Narrow</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

In Table 2.1, sensor accuracy is defined as the degree to which the measurement system indicates the true temperature being measured. Repeatability is the sensor’s ability to repeatedly produce the same reading during successive measurements of a reference temperature.

2.1.3 RTD: Sensing principle

RTDs operate based on the ‘Thermoresistivity’ principle. The rise in temperature causes the flow of electrons to increase in the metal thereby leading to an increase in its electrical resistance $R$. Similarly the metal’s resistance decreases as temperature decreases. This linear temperature-resistance relationship can be monitored only by the use of a measuring current $I$. Therefore RTDs are passive devices which require an external power source. Consequently the $I^2R$ heating due to the external current can raise the temperature of the RTD above the ambient temperature causing ‘self-heating’ of the sensing element. RTD resistance is unique to every metal it is composed of and can be determined theoretically using the Equation (2.1). $R$ is the resistance of the metal wire in Ω while $\rho$ is the resistivity in Ωm, $L$ is the length of the metal wire in m and $A$ is the cross-sectional area of the wire in m².

$$R = \frac{\rho L}{A} \quad (2.1)$$
Although an RTD’s transfer function is the most linear when compared to that of TCs and thermistors it is not perfectly linear. The ‘Callendar-Van Dusen’ Equation (2.2) is used to model the function accurately for measuring wide temperature ranges. The constants A, B and C are dependent on the material properties of the RTD. \( R_t \) is the resistance at temperature \( t \) and \( R_0 \) is the nominal resistance at 0\(^\circ\)C.

\[
R_\text{t} = R_0(1 + At + Bt^2 - 100Ct^3 + Ct^4) \quad (2.2)
\]

In this thesis, since the RTD is used only for sensing temperatures ranging from 23 – 100\(^\circ\)C the Callendar-Van Dusen equation can be reduced to a linear relationship \[232, 111\] as seen in Equation (2.3).

\[
R_\text{t} = R_0(1 + \alpha(t - t_0)) \quad (2.3)
\]

where \( \alpha \) is the temperature coefficient of resistance (\$/\(^\circ\)C) or TRC and \( R_0 \) is the RTD resistance (\( \Omega \)) at a reference temperature \( t_0 \). An ideal RTD will exhibit a linear temperature relationship as seen in Figure 2.1.

![Figure 2.1: RTD transfer function: The change in resistance (\( \Delta R \)) is directly proportional to change in temperature (\( \Delta T \)) and material property \( \alpha \).](image-url)
RTD self-heating

Self-heating is also termed electric joule-heating in resistors. Joule-heating is generated in a resistance $R$ when a current $I$ is passing through a resistor dissipating a certain amount of heat energy $P$ as some of the electrical energy gets converted to heat energy. The current imparts energy to the electrons causing them to accelerate through the bulk of the material. When these electrons collide with the heavy ions the kinetic energy released is converted to heat energy. Self-heating error is characterized by the equation (2.4) where $P = I^2 R$ in watts and heat dissipation constant (usually between 20 – 100 mW/°C) is assumed to be 50 mW/°C

$$Self - heating\ error = \frac{P}{Heat\ dissipation\ constant}$$

(2.4)

Since self-heating errors are inevitable in resistive sensors optimum sensor current must be set below the point where the self-heating produces a measurable increase in resistance [79].

2.1.4 Material selection

The thermo resistive characteristic of RTDs depends on the type of metal or metal alloy they are made of. Most commercial RTDs are made from platinum, copper, nickel or balco (nickel-iron alloy) [256] which have a high melting point and can withstand corrosion damage. Platinum RTDs are most commonly used due to their inertness to chemicals and other environmental factors. However nickel has several advantages over the other metals. One, nickel costs $\approx 250$ times less than precious metals such as platinum [194] making it optimal for creating disposable biosensors. Two, ‘self-heating’ of the RTD as described in 2.1.3 will be lower in metals with higher $\alpha$ value [256]. This is because the excess temperature acquired by the RTD during self-heating will be dissipated by the body of the metal more efficiently to its surroundings when its $\alpha$ is higher. Table 2.2 lists the $\alpha$ values of common metals [256]. The temperature coefficient of

<table>
<thead>
<tr>
<th>Metal</th>
<th>$\alpha$ (%/°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum</td>
<td>0.385</td>
</tr>
<tr>
<td>Copper</td>
<td>0.4</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.618</td>
</tr>
<tr>
<td>Balco</td>
<td>0.518</td>
</tr>
</tbody>
</table>
resistance (TRC) or $\alpha$ as defined in Figure 2.1 is the average resistance change per °C change in temperature with respect to initial resistance $R_0$ and is expressed in terms of $\%$/°C. Three, the percentage change in $\alpha$ per °C is the highest when compared to the other metals. In other words nickel exhibits twice as much overall resistance change than platinum for the same temperature change sensed. Lastly, from a fabrication perspective unlike platinum [20, 52, 51] nickel does not require an adhesion layer during deposition on glass or other substrates [129]. For reasons stated above nickel was chosen as the electrode material for patterning the heaters and sensors throughout this thesis.

2.2 Heater and RTD Design: Finite Element Analysis

Prior to the actual fabrication of these microelectrodes their physical design was first optimized using finite element modeling (FEM). The use of FEM simulation assisted in predetermining much of the microelectrode layout and geometrical parameters thus helping in minimizing development costs. The main objectives of design optimization using FEM simulation were

1. To achieve uniformity in temperature distribution around the heater
2. To determine the optimal proximity of the RTD to the heater
3. To evaluate the physical design of the bioreactor.

COMSOL Multiphysics 3.4 (Stockholm, Sweden) was used to evaluate thermal profiles of the microelectrodes and bioreactor.

A variety of microelectrode and bioreactor chamber designs were developed during the course of this project. To demonstrate the FEM design methodology applied in developing the DTA microcalorimeter one representative model each of the microelectrodes and bioreactor are presented in this section. Detailed descriptions of the device designs have been discussed in subsequent chapters. Briefly, a nickel heater and RTD pair are modeled on the bottom-side of a glass coverslip. A cylindrical bioreactor chamber made of polydimethylsiloxane (PDMS) which is coupled for fluid handling is modeled along with the heater. Thermal properties of the different materials used in the construction of the microcalorimeter are summarized in Table 2.3.

2.3 Heater Design

A 2D schematic of the heater is shown in Figure 2.2. The thickness of the film was neglected and modeled as a 2D boundary as it was a few orders of magnitude less than the thickness
Table 2.3: Device Construction Materials and Their Properties

<table>
<thead>
<tr>
<th>Device component</th>
<th>Material</th>
<th>Thermal conductivity (W/mK)</th>
<th>Density (kg/m³)</th>
<th>Specific heat capacity (J/kgK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heater, RTD</td>
<td>Nickel</td>
<td>90.7</td>
<td>8900</td>
<td>445</td>
</tr>
<tr>
<td>Bioreactor</td>
<td>PDMS</td>
<td>0.18</td>
<td>1030</td>
<td>1100</td>
</tr>
<tr>
<td>Substrate</td>
<td>Glass</td>
<td>1.11</td>
<td>2200</td>
<td>830</td>
</tr>
<tr>
<td>Fluid sample</td>
<td>Water</td>
<td>0.58</td>
<td>1000</td>
<td>4187</td>
</tr>
</tbody>
</table>

Figure 2.2: Heater geometry: The width of the traces and line-spacing was 30 µm while the thickness of the nickel was 200 nm on a 100 µm thick glass substrate. The total length of the nickel coil was ≈ 12 mm imparting a resistance of ≈ 150 Ω.

of the glass. Modeling the film in 3D will exponentially increase the number of mesh elements at the interfacial boundary costing immense computational memory requirements. The general heat transfer mode within the heat transfer module in COMSOL was used to simulate the temperature distribution profile of the heater. The heat transfer from the heater was modeled using the in-built ‘highly conductive layer’ feature and the power dissipated per unit area was calculated using the Equation (2.5)

\[
P_{\text{heater}} = (T_{\text{film}})(P_d) \tag{2.5}
\]
where $P_{\text{heater}}$ is the power generated per unit area of the heater in W/m²K, $T_{\text{film}}$ is the film thickness in nm, $P_d$ is the power density of the heater in W/m³K. The heat lost to the air through natural convection was assumed to be 50 W/m²K. The resistive heating within the film was modeled using the ‘shell conductive media DC’ mode within the AC/DC module. The heat generated by the heater in W/m² due to the input voltage was calculated using the Equation (2.6)

$$Heat = (T_{\text{film}})(E)(\kappa)$$

(2.6)

where the input electric potential $E = 1$ V and $\kappa$ is the electrical conductivity of nickel in siemens/meter (S/m). All boundary conditions were forced as heat flux to emulate real-world conditions i.e the device was exposed to air on all sides and was without encapsulation. All subdomain parameters were set from the material properties seen in 2.3. The Figure 2.3(a) shows the 2D solution of the heater temperature distribution. Coarse sized Lagrange triangular

![Figure 2.3: Heater temperature distribution: The central bar shows the color scheme of the representative temperature in °C. (a)The 2D map shows that the temperature distribution is radially uniform around the coil diameter (b)The 3D rendition shows that the front-side of the glass also experiences similar temperature distribution without major heat losses.](image-url)
pre-defined mesh elements were used to compute an approximate solution in the transient mode for 1 s. Transient analysis was performed using a non-linear solver and 126485 degrees of freedom to determine the temperature distribution of the heater coil after 1 s. The 3D solution in Figure 2.3(b) was obtained by extruding the geometry of the coil from 2D to 3D while glass parameters were forced throughout the thickness except on the coil lying on the bottom surface. The 3D model illustrates that the heat loss through radiation and conduction were minimal and that the non-nickel side of the glass also experiences similar heat intensity and temperature distribution as the nickel-side.

2.4 Relative RTD Position with Reference to Heater

The effect of the heater after integration with all the components of the microcalorimeter was modeled to evaluate the optimal position of the RTD from the heater. A 2D schematic of the RTD positioned 400 µm from the heater was shown in Figure 2.5. The heater and RTD were modeled as line electrodes within a water filled cylindrical PDMS bioreactor chamber. It was assumed that the water completely fills the chamber without no air spaces. Figure 2.4(a) represents the cross-sectional geometrical and material properties of the model while (b) represents the boundary conditions applied on each interface. The outer boundaries of

![Figure 2.4: Optimal RTD position with reference to heater. (a)Schematic of materials and their respective geometries: the PDMS and glass were assumed to be physically bonded together to allow heat exchange through their interface. (b)Corresponding boundary conditions of microcalorimeter interfaces: All outer boundaries of the device were general heat flux interfaces except the nickel-side of the glass substrate to allow heat dissipation through the glass thickness. (Note: Figure not to scale).](image)

PDMS and glass were heat flux boundaries allowing them to freely exchange energy with the surrounding which emulates the chip being exposed to the environment without insulation. The subdomain parameters applied to the individual device components were as listed in Table
2.3. The model was meshed with 657 coarse-sized Lagrangian triangular elements. Steady state analysis was performed with 1388 degrees of freedom. The surface temperature distribution of the device is as seen in Figure 2.5(a). The general heat transfer module was used to model the heat transfer from the heater to the individual device components with a steady temperature of 40.85°C assigned to the heater while the rest of the outer boundaries of the device were modeled as heat flux surfaces. The inner boundaries or the interfaces between PDMS-water, PDMS-glass and glass-water were all assigned the ‘continuity’ boundary condition which equalizes heat fluxes from the individual materials at the interface. The cross-sectional temperature plot from the middle of the bioreactor chamber as seen in Figure 2.5(b) shows that the temperature uniformity is not maintained beyond the heater area and that the sensor only marginally experiences the exponentially decreasing temperature. This observation suggests that positioning the RTD at any planar distance from the heater will not allow it to sense the true temperature profile as emitted by the heater. In retrospect we observe from Figure 2.3 that the area within the heater would be a position from where the RTD could experience uniform heater temperature. Based on the evidence presented above the RTD was placed concentrically within the heater for optimal sensing as seen in Figure 2.6. Such a design idea was employed for all device generations throughout the project with variations in the base resistances depending on the sample size and position.

Figure 2.5: Optimal RTD proximity to heater: (a) Steady-state surface temperature plot of the cross-section of device with the color schemed temperature chart representation in °C. The heat transfer coefficient $h$ was assumed to be 50 W/m$^2$K [270] (b) Cross-sectional temperature profile: The dotted line along the chamber represents the line along which temperature was plotted as a function of the device length.
2.5 Bioreactor Design

Similar to the uniform temperature distribution around the heater as seen in Figure 2.3 it was important to create temperature profiles within the chamber with minimal gradients. The heater resulted in changing the density of the fluid inside the chamber thereby setting up convective currents. This fluid motion ensured continuous mixing and the sample within was subjected to uniform temperatures throughout the volume of the chamber. Similarly in a droplet sample (refer to Chapter 5) mixing was enhanced through ‘marangoni flow’ [208]. This type of circulatory flow accelerated sample homogenization via mixing of the hot and cold parts of the liquid thus reducing the overall time to reach steady-state.

2.5.1 Marangoni effect

Surface tension is a fundamental property of a liquid that imparts a contractile or tensile force on its surface [73, 262]. Surface tension is a function of concentration and temperature [21, 66]. Temperature gradient along the interface creates convective instability causing motion in the bulk liquid [6, 157]. Such a surface tension dependent motion is termed ‘thermocapillary effect’ or the ‘marangoni effect’. Figure 2.7 illustrates the mechanism of Marangoni flow. A heat source heats up one side of the water droplet surface. This heat creates a thermocapillary tangential stress on the surface that propels the liquid away from the hot spot. In other words, the heat decreases surface tension at the hot spot and the surface molecules begin moving toward the cooler region of the droplet where the surface tension is higher. Such surface motion eventually causes bulk motion within the liquid setting up convective flows within the liquid [212].
Figure 2.7: Marangoni effect: The heat source causes a thermal gradient in the liquid resulting in the movement of surface molecules from a region of lower surface tension (hot area) to higher surface tension (cool area). Surface motion eventually induces bulk motion within the liquid.

2.5.2 Chamber design

Several geometries of chambers were modeled with different heater positions. Through these computational models we concluded that the temperature distribution in the chamber was dependent on the following factors:

1. The height and width of the chamber with respect to the heat source
2. The size and position of the heat source

It was determined that tall chambers produced irregular heating patterns within the chamber and took longer time to reach steady-state. An ideal chamber would show minimal thermal gradients throughout its volume and also contain small sample volumes without complicating fabrication protocols or causing sample adsorption due to reduced surface to volume ratio. The model described in this section was the final optimized chamber design universally employed to fabricate the bioreactor chamber for subsequent devices.

A cylindrical chamber 250 μm tall and 2 mm in diameter was designed to contain 0.8 μL of sample. A schematic of a 2D cross-sectional area of the chamber is shown in Figure 2.8 with (a) showing the physical dimensions of the chamber and (b) showing boundary settings assigned on each surface. The heat source applied as a 1 mm line heater was assumed to be transmitted through the thickness of the glass coverslip. Heat flux boundaries emulate a real-world situation where the device is exposed to ambient air without insulation. A heat flux value of 10 W/m² was used to model the heat losses through the chamber walls.
Figure 2.8: Optimal bioreactor geometry: (a) Schematic representation of 2D cross-section of cylindrical bioreactor chamber. The heat source is representative of the heat transmitted through the 100 µm glass thickness (b) Schematic representation of boundary settings. Temperature 1 is set as a constant initial value of 300 K.

Table 2.4: Water: Thermal and Material Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>Temperature of heat source</td>
<td>40°C</td>
</tr>
<tr>
<td>C&lt;sub&gt;p&lt;/sub&gt;</td>
<td>Specific heat</td>
<td>4187 J/kgK</td>
</tr>
<tr>
<td>k</td>
<td>Thermal conductivity</td>
<td>0.58 W/mK</td>
</tr>
<tr>
<td>α</td>
<td>Thermal expansion coefficient</td>
<td>214 (10&lt;sup&gt;-6&lt;/sup&gt;/K)</td>
</tr>
<tr>
<td>ρ</td>
<td>Density</td>
<td>1000 kg/m&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>η</td>
<td>Dynamic viscosity</td>
<td>0.653 (10&lt;sup&gt;-3&lt;/sup&gt;Pa.s)</td>
</tr>
<tr>
<td>g</td>
<td>Acceleration due to gravity</td>
<td>9.8 (m/s²)</td>
</tr>
</tbody>
</table>

Fluid samples used during experimentation ranged from isotonic buffer solutions to buffer-based DNA suspensions. However, water was modeled here for the sake of simplicity with subdomain properties [286, 155] as listed in Table 2.4. Triangular Lagrange elements were used to coarsely mesh the chamber to generate an approximate solution. 127 mesh elements were used along with 952 degrees of freedom for transient analysis of the chamber. The 2D simulation results are as seen in Figure 2.9. The temperature distribution within a trapezoidal and cylindrical chamber shown in Figure 2.10 are also presented for comparison. The 2D cross-sections of each of the chambers were first sketched in COMSOL. The trapezoidal chambers as seen in Figure 2.10(a) are usually products of bulk silicon etching. The 2D simulation for each type of chamber in Figure 2.10 was obtained by assigning matching parameters for each simulation. Post-processing of the 2D simulation included using both surface and arrow plots superimposed on the same result. While the surface plot denoted temperature values the arrows were vector representations of the amplitude and direction of the velocity flow of the fluid. It
Figure 2.9: Optimal bioreactor geometry: The heat source is representative of the heat transmitted through the 100 µm glass thickness. Arrows were representative of velocity field while the surface represents the temperatures described in the scale on the right (°C). Transient analysis was performed for 10 s.

Figure 2.10: Optimal bioreactor geometry: The heat source is representative of the heat transmitted through the 100 µm glass thickness. Arrows were representative of velocity field (a) Trapezoidal bioreactor chamber: The top corners of the chamber do not undergo proper mixing (b) Square bioreactor chamber: Higher temperature gradients within the volume of the chamber.

can be observed that the trapezoidal chamber exhibits wider temperature gradients than the cylindrical chamber seen in Figure 2.9. Based on this evidence a tall cylindrical chamber with
an aspect ratio (1 : 8 :: width : height) similar to the short cylinder seen earlier would show even higher temperature gradients due to the distance of the heater from the top of the chamber. Hence a square chamber (cylinder) has been presented in Figure 2.10(b) for comparison with the cylindrical chamber (2.9). A $1 \times 1$ square cross-sectional area was selected instead of a $2 \times 2$ to reduce computational time.

The comparison between various chamber geometries shows that internal geometries with minimal distance variation from a central axis such as that of cube, cuboid or cylinder produce more uniform temperatures within the chamber when compared to boundaries such as the trapezoidal chamber. The surface profile shows temperature distribution while the arrows show the velocity field distribution once the convective currents take to effect due to the momentary density gradients induced within the chamber. The lengths of the arrows represent the strength of the velocity at that position. The fluid motion within the cylindrical chamber helps in the thorough mixing of the sample during the heating process ensuring uniform exposure to the thermal source. Therefore a cylindrical chamber would offer the sample the fastest homogenization rates and can be easily manufactured using soft lithography techniques.
Chapter 3

Generation I: Glass-PDMS Hybrid

A glass-based device with inbuilt heating and sensing elements was integrated with polydimethylsiloxane (PDMS) microfluidics to create the first generation device in the series of microcalorimeters designed through the course of this project. We report a simple and step-by-step approach toward eliminating bubble formation and simultaneously limiting evaporation during device operation. This novel technique of rendering the device virtually bubble-free even at internal temperatures $> 100^\circ C$ can be incorporated in common types of PDMS based microfluidic chips involved in heating of liquid samples. As a proof-of-concept a 20-mer dsDNA (double-stranded DNA) sample labeled with a fluorescent dye is melted by heating the sample while its melting signature is captured optically. The on-chip heater and temperature sensor are experimentally characterized for performance, accuracy and sensitivity. In addition we present a method of introducing nanoliter volume droplets of DNA into the PDMS microreactor chamber.

3.1 Background

The advent of microfluidic systems since the invention of polydimethylsiloxane (PDMS) has revolutionized the field of biosensor development. George Whitesides group [74, 296, 252, 100] have popularized the use of PDMS in a variety of applications. PDMS-based biosensors have since expanded their applications in lifescience, drug chemistry, medical device technology and medicine [138, 283, 151, 102] to an extraordinary degree. PDMS serves as the common substrate material in a variety of applications for conducting miniaturized bench-top laboratory procedures [184, 252, 150, 100]. Polymers such as PDMS offers a host of advantages for use in lab-on-a-chip systems such as ease of fabrication, rapid prototyping, ease of surface functionalization, biocompatibility, cost-effectiveness and optical transparency with low autofluorescence [5, 243, 121, 24, 216, 215]. PDMS is also gas-permeable [42] which makes it an excellent substrate for performing cell culture experiments [158]. The porosity advantage of PDMS that
allows for exchange of gases to take place in order for mammalian cells to survive also allows for evaporation of liquid samples \cite{197,269}. The loss of sample volume through evaporation might also involve formation of gas bubbles.

### 3.1.1 Theoretical estimation of thermal time constant

Thermal time constant (\(\tau\)) is defined as the time required by the body to reach 63\% of the total difference between its initial and final temperatures when subjected to a step function change in temperature. Predicting the time constant is important as it can be used as a tool to estimate the temperature of the body at a given point in time and enables the user to understand the optimal speed for thermal operation of the device. The thermal time response of the chamber was calculated in order to characterize its response to a step input. The theoretical thermal time constant \(\tau\) for a lumped system was calculated using the equation (3.1).

\[
\tau = \frac{\rho C_p V}{hA_s} \quad (3.1)
\]

Assuming that the cylindrical chamber with a surface area \(A_s\) \(\approx\) 8 mm\(^2\) and volume \(V\) \(\approx\) 1 \(\mu\)L contained water with specific heat capacity \(C_p\) = 4181 J/kgK and density \(\rho\) 1000 kg/m\(^3\) the body of water would lose heat to the ambient air proportional \cite{270} to the heat transfer coefficient \(h\) 40 W/m\(^2\)K, the thermal time constant \(\tau\) was calculated to be 13 s.

### 3.2 Bubble Formation and Evaporation

Bubble formation within microfluidic channels during on-chip heating operations is a routine occurrence \cite{275,16}. Intentional bubble generation and control can benefit applications such as the preparation of ultrasound contrast agents \cite{209,115}, actuation \cite{95}, molecular beacons for DNA sensing \cite{67}, on-chip manipulation of microscopic objects \cite{164,53} and microorganisms \cite{297,138}, designing vehicles for drug molecule synthesis and targeted drug delivery \cite{48,114}, sorting for enrichment of particles \cite{282} and on-chip mixing \cite{273}. However, unwanted bubbles caused during the operation of microfluidic devices can negatively affect experimental results \cite{175,75}. At the macroscale, bubble formation does not degrade device performance, but at the microscale bubbles can decrease the signal to noise ratio or even render the device inoperable \cite{263,168,134}.

Bubbles can arise from discontinuities in the macro-micro interfaces, gas permeability of PDMS, drawbacks in microfabrication methods resulting in dead volumes which trap gases \cite{168}, rapid evaporation or the formation of nucleation sites due to localized on-chip heating.
During nucleation, bubbles form as the solution temperature rises, causing the solubility of dissolved gases to decrease, thus promoting bubble formation. The combination of PDMS bioreactors for holding liquid samples and on-chip microheating elements invariably encourages bubble formation. Thus a significant amount of effort has been invested in devising methods for eliminating bubbles and reducing evaporation during on-chip biomolecular processes such as polymerase chain reaction (PCR) and melting curve analysis (MCA).

### 3.2.1 Bubbles during PCR

Many research groups have designed various techniques to address the issue of bubble-formation. Pisignano et al. designed a method to reduce evaporation during PCR by embedding glass microchannels inside PDMS microreactors [217], but observed bubbles when the temperature was increased to 95°C. While some groups removed the unwanted bubbles by creating complex microfluidic traps [45, 253, 170], other groups limited bubble formation by degassing the solutions [192], coating PDMS with parylene [251] or bubble extraction by coating PDMS with teflon [134], cladding air pockets with more PDMS which have potential for bubble initiation [168], pre-wetting and sealing the microreactor areas with the PCR sample [202], capillary wetting [226], increasing chamber pressure by flushing with fluorinated oil [198] or nitrogen [168], manually deforming the PDMS chamber to force out bubbles [118, 168] and utilizing commercially available off-chip microvalves to control chamber pressure [34]. While bubble traps suffer from increased gas pressures at elevated temperatures, PDMS cladding techniques continue to allow outgassing of the materials. Pressurization on the other hand requires that the bond strength of the device and the substrate rigidity be high.

### 3.2.2 Bubbles during MCA

Regardless of the type of detection used for DNA analysis during MCA, bubble-formation has been known to negatively affect [214] thermal profiling. Fiche et al. [88] employ surface plasmon resonance imaging for chip based MCA and suggest degassing the experimental solution before thermal cycling to avoid bubble formation. Other groups have primed microfluidic channels via negative pressure [237] for conducting chemical gradient mediated MCA or have cautiously introduced [49] the sample into the device followed by sealing on both ends of the tubing to avoid bubble formation. Similarly when determining the melting transition of DNA purely calorimetrically water vapor bubbles can be detrimental to the experiment [190]. Gas bubbles which form near the heating elements due to joule heating not only disrupt the operation of the device but also have the potential to yield false positives due to their inherent characteristic of bearing lower temperatures than the surrounding liquid sample.

In this chapter we report the fabrication and characterization of a cost effective microfluidic
MCA device with a rapid response time and low sample volume consumption. A novel contribution of generation I devices is the technique of pressurizing the reaction chamber without affecting the experimental sample and as a means to increase signals to noise ratio at the data acquisition end due to sample containment without interference of bubbles or rapid evaporation.

3.3 Materials and Methods

3.3.1 Experimental setup

A block diagram of the experimental setup is shown in Figure 3.1. The chamber was made of optically transparent PDMS, parylene and glass. A syringe pump was used to inject sample into the reaction chamber. Once the chamber was full, the outlet tubing was pinched closed and the syringe pump was used to pressurize the chamber to reduce bubble formation during heating. The resulting increase in chamber pressure was recorded using a pressure sensor (DelTran 400, 39

Figure 3.1: System-level block diagram showing the connections of the microfluidic device to the optics, flow control and measurement electronics.
Utah Medical, USA) connected in series with the syringe pump and the device. The microfluidic device was mounted on an epi-fluorescence microscope stage to help visualize dsDNA melting. The on-chip heater and sensor were controlled via a custom Labview program (National Instruments, TX, USA). The temperature sensor data were used as feedback to the heater power supply to actively control chamber temperature. Prior to dsDNA injection the tygon tubing, channels and the rest of the microfluidic chip were filled with 1×TE buffer solution to maintain a bubble-free, closed system environment.

The chip was mounted on a custom-made acrylic jig. The individual parts of the acrylic jig were cut out using a CO\textsubscript{2} laser cutter and assembled to form a contraption as seen in Figure 5.6. The jig was designed to hold 0.25'' long gold-plated, spring loaded pogo-pin connectors (Mill-Max Connections, New York, USA). The connectors were used to create a solderless, shock-proof connection with the heater and RTD. The heater was connected to a 3-decimal precision power supply operated in constant-current mode. RTD resistance was measured with a 6½ digit precision digital multimeter (DMM) in 4-wire mode (Keithley 2010, Ohio, USA). The entire setup was exposed to ambient room temperature without an insulating enclosure.

![Image of acrylic jig](image)

Figure 3.2: Photograph of acrylic jig for mounting glass-PDMS chip. Inset shows pogo-pins making electrical contacts with the chip’s probe pads.
3.3.2 Buffers and reagents

dsDNA

Validating the performance of the device requires the use of dsDNA samples with a known melting point. Hence a 12-mer dsDNA sample (Integrated DNA Technologies, Iowa, USA) with the sequence 5′ - CGCGAATTCGCG was used to acquire melting curves. Nuclease-free 1× TE buffer (30 mM Hepes, pH 7.5, 100 mM potassium acetate, IDT, USA) was used for initial resuspension of the anhydrous DNA pellet to make a 500 µM stock solution. Four dsDNA concentrations (742, 10, 1 and 0.1 µM) were prepared by serial dilution of the stock solution.

EvaGreen

EvaGreen dye (Biotium Inc., California, USA) was used for the optical detection of DNA melting. EvaGreen is an intercalating dye comprising two monomeric DNA-binding dyes linked together by a flexible spacer. EvaGreen has an excitation maximum at 500 nm and an emission maximum at 530 nm when bound to dsDNA. The melting point of the dsDNA sample as reported by the supplier was 46°C. Approximately 5 µL of 20× EvaGreen dye was mixed with each of the 100 µL dsDNA aliquots from 3.3.2, stirred and allowed to rest for 10 min at room temperature before testing. Figure 3.3 shows the active and inactive states of EvaGreen while in solution. The dye construct is designed such that the dye molecules (depicted in glowing green) become available and get activated only in the presence of dsDNA. When dsDNA melts to form ssDNA, the dimeric dyes get inactivated and bind together in a looped position without fluorescence emission. When the dsDNA becomes available again, they dissociate and bind to dsDNA resulting in fluorescence. This concept of the dye becoming available only in the presence of dsDNA is termed as ‘release on demand’ mechanism. Most multi-cationically charged intercalating dyes are highly positive and hence could bind favorably with both ssDNA and dsDNA [266]. Such poor selectivity is due to the electrostatic interactions between the dye molecules and DNA [178]. Although information on the exact structure of Evagreen is unavailable research groups have demonstrated the it has high selectivity to dsDNA and low affinity to ssDNA [76, 241].

Microscope setup

Fluorescence images of the melting dsDNA within the chamber were captured using an inverted fluorescence microscope (Olympus IX71, PA, USA) fitted with a cooled monochrome CCD camera (ORCA-ER, Hamamatsu, Japan). The EvaGreen dye tagged to dsDNA within the PDMS bioreactor was imaged with a 500 ms exposure time and a FITC filter cube. One image was captured every 5 s for 150 s.
Figure 3.3: Illustration of EvaGreen dye behavior in solution: The intercalating dye incorporated into the dsDNA helical grooves is deactivated when the dsDNA denatures into ssDNA.

3.3.3 Fluorescence data analysis

Fluorescence data from the chamber was recorded simultaneously with resistance measurements from the RTD. The RTD data was used to map the chamber temperature during the scan. The recorded fluorescence intensity measurements were analyzed using ImageJ software (NIH, USA). Each experiment was recorded as an image stack of 31 images and stored as high resolution files in the tagged image file format (TIFF). The following steps were performed to convert the raw image data into discrete fluorescence intensity measurements. A circular region of interest (ROI) was carefully positioned within the radius of the RTD without including any part of the RTD. This ROI which constituted $\approx 3000$ pixels was linked to the image stack. Discretized average intensity data obtained after compiling the image stack was used as the core fluorescence data for each experiment. Three sets of such raw data were obtained from three trials for every DNA concentration. Each set was normalized between 0 and 1 by using the formula seen in (3.2) and subsequently averaged to obtain one final normalized mean curve. In this equation $D_{\text{norm}}$ is the resultant normalized datum, $D_i$ is the corresponding datum being normalized, $D_{\text{min}}$ is the least value datum and $D_{\text{max}}$ is the highest value datum in each trial set. The first
derivative of the normalized curve was plotted to understand the sensitivity of the scan. In other words the ‘differential’ of the curve was used to test the amount of change of temperature with respect to the change in input power (in terms of sequential image stepping). A general differential was calculated using the formula (3.3) where \( m \) is the slope of the line, \( \Delta F \) is the change in normalized fluorescence intensity and \( \Delta i \) is the change in the image sequence which was constant at 1 as the acquired images were sequential.

\[
D_{\text{norm}} = \frac{D_i - D_{\text{min}}}{D_{\text{max}} - D_{\text{min}}} \tag{3.2}
\]

\[
m = \frac{\Delta F}{\Delta i} \tag{3.3}
\]

### 3.3.4 Device fabrication

**Fabrication of heater and RTD**

A schematic representation of the process flow is shown in Figure 3.4. Glass coverslips measuring 100 \( \mu \)m in thickness and 1” \( \times \) 1” in area were cleaned with a commercially available mixture of hydrogen peroxide and sulphuric acid (NanoStrip, Cyantek, CA, USA). The glass coverslips were coated with 1.5 \( \mu \)m of the image-reversal photoresist AZ 5214 E-IR (Capitol Scientific Inc., TX, USA) by spinning at 4000 RPM for 45 s. The coverslips were then soft-baked at 95\(^\circ\)C for 2 min to improve adhesion to the glass surface and to drive out excess solvent in the photoresist. The photoresist layer was patterned via a photomask through an i-line optical filter with a minimal exposure dose of 30 mJ/cm\(^2\) to form trapezoidal exposed areas within the photoresist thickness. An image reversal bake step was performed after the exposure on a 115\(^\circ\)C hot plate for 1 min which rendered the exposed areas cross-linked to form a developer insoluble and light-insensitive substance while the unexposed areas remained chemically similar to normal photoresist. A flood exposure of 600 mJ/cm\(^2\) following the reversal bake step ensured previously unexposed areas became completely soluble. The substrate was then developed in Microposit metal ion-free CD-26 developer (Rohm and Haas Electronic Materials LLC, MA, USA) for 30 s, leaving a negative image of the mask pattern with retrograde sidewall profiles to facilitate good lift-off processing. An electron beam evaporator was used to deposit a 200 nm thick film of nickel (Kurt Lesker, 99.99% pure) on the patterned glass substrates. The nickel-coated substrates were then treated with acetone to lift off nickel from the unpatterned areas, rinsed with deionised water, and dried with nitrogen. The sensor was designed to have a nominal resistance of 100 \( \Omega \) at room temperature, while the heater resistance was designed to be 400 \( \Omega \).
Figure 3.4: Process flow for microelectrode fabrication using silicon photolithography principles: (1) Clean glass coverslip (2) Spin coat 1.5 µm thick photoresist on surface (3) Soft bake at 95°C (4) Expose to UV light through a photomask to yield (5) Underexposed portions in the photoresist (6) Image reversal bake cross-links exposed photoresist pattern (7) Flood expose without a mask to expose remaining photoresist (8) The flood-exposed photoresist pattern is developed (9) Nickel is evaporated on the substrate surface (10) Remove photoresist using a solvent.

Chamber fabrication

Standard soft lithography techniques as seen in Figure 3.5 were used to create PDMS chamber of 1 µL volume. PDMS microfluidic molds could be prototyped using a SU-8 master. A silicon substrate 3” diameter and thickness 500 µm (University wafer, MA, USA) was cleaned thoroughly with piranha solution (70% sulfuric acid, 30% hydrogen peroxide), rinsed with deionised water and dried using nitrogen. SU-8 2100 negative photoresist (Microchem, MA, USA) was spin coated at 800 rpm for 30 s to form a 250 µm layer. The substrate was then soft baked for 5 min at 65°C and 10 min at 95°C to drive out solvents and enhance exposure. The substrate was exposed to UV light to impart a dosage of 700 mJ/cm² for patterning the photoresist. A post exposure bake step was performed for cross-linking the exposed photoresist. The substrate was baked for 3 min at 65°C and transferred to a 95°C hotplate for 10 min. SU-8 developer
Figure 3.5: Process flow for PDMS microfluidics fabrication using soft lithography principles:
(1) Clean silicon wafers (2) Spin coat 250 µm thick SU-8 photoresist on surface (3) Soft bake at 95°C (4) Expose to UV light through a photomask to yield (5) Exposed portions in the photoresist (6) Perform a post exposure bake at 95°C to cross-link the exposed regions of photoresist (7) Develop photoresist to remove unexposed photoresist areas. PDMS Microfluidics (1) Start with SU-8 master from earlier process (2) Pour PDMS prepolymer on the master and cure (3) Peel off the cured PDMS from the master.

(Microchem, MA, USA) was used to dissolve the areas that did not cross-link. The substrate was rinsed with water and dried using nitrogen. Such a ‘master’ substrate with patterned SU-8 was used for the rapid prototyping of PDMS molds.

PDMS microfluidics were created by mixing the PDMS elastomer and curing agent (Sylgard 184, Dow Corning, MI, USA) in a 10:1 ratio. This prepolymer was poured on the SU-8 master and cured on a hotplate at 85°C for two hours. The cured PDMS mold was peeled off of the substrate and the SU-8 master could be reused for prototyping more PDMS chambers.

Device assembly

Figure 3.6 is an optical micrograph of the top view of the device showing the heater and RTD enclosed within the PDMS chamber. The inner surfaces of the PDMS chamber and channels were coated with 10 µm of parylene using an SCS Parylene Coater (PDS 2010 Labcoater, Indiana, USA). Non-porous and commonly available gift-wrapping tape was used to mask cer-
Figure 3.6: Optical micrograph of device: The PDMS chamber was bonded irreversibly on the nickel-free side of the glass substrate. The trace width and spacing of the heater and RTD were 30 µm.

Figure 3.7: Device overview in 3D: Schematic showing the different layers of a fully assembled device. The nickel heater and RTD on the nickel-free side of the substrate were separated from the PDMS chamber by the 100 µm thickness of the glass coverslip.
tain areas on the PDMS surface that would later come in contact with the glass coverslip for bonding. The parylene-coated PDMS chamber was positioned to enclose the heater and RTD within the area of its outer diameter and irreversibly oxygen-plasma bonded to the nickel-free side of the glass substrate using a basic plasma cleaner (Harrick Plasma, NY, USA). Finally, an additional 10 µm parylene layer was deposited on the outside of the assembled device and parylene-coated tygon tubing (outer surfaces only) were inserted to complete the device. Figure 3.7 shows a 3D view of a fully assembled device. The bioreactor chamber had inlet and outlet channels to facilitate sample loading through tygon tubing.

3.4 Results

3.4.1 RTD performance

![RTD performance graph](image)

Figure 3.8: RTD calibration: A representative plot of RTD resistance as a function of temperature. Resistance was measured at non-uniform temperature intervals because of the coarseness of the temperature knob on the oven. The sensor shown here had an $\alpha = 0.0032 \degree C^{-1}$. All other sensors used in experiments had similar $\alpha$ values.

A coverslip containing heater and RTD was placed inside an oven and the temperature was manually ramped from 24 – 100°C while recording the RTD resistance. PDMS microfluidics were not included in the calibration experiment. A mercury thermometer was used to accurately measure the oven temperature. The oven was allowed to reach steady-state prior to each RTD measurement. Figure 3.8 shows a representative RTD response with good linearity ($R^2 = 0.998$)
over the temperature range tested. The linear fit was used to extract the temperature coefficient of resistivity alpha ($\alpha$) using the equation (3.4)

$$\alpha = \frac{R_f - R_i}{R_i \Delta T}$$

(3.4)

where $R_f$ is the RTD resistance at 100°C, $R_i$ is the resistance at 23°C and $\Delta T$ is the difference between final and initial temperatures. The calculated $\alpha$ value was 0.0032°C$^{-1}$, which is lower than the published value of 0.0065°C$^{-1}$ [211].

We hypothesized that contamination inside the electron beam evaporator led to the deposition of a nickel alloy instead of pure nickel yielding a lower $\alpha$ value than expected.

3.4.2 Thermal time constant

In order to experimentally determine the thermal time constant of the chamber, we applied a range of excitation current step inputs to the on-chip heater and the resulting chamber

![Figure 3.9: The RTD was used to measure the change in chamber temperature in response to a current step input to the heater. The heating time constant, the time required for the chamber to reach 63% of its final temperature, ranged from 18-20 s, depending on the excitation current amplitude. Each curve is the average of 3 trials ($n=3$).](image-url)
temperature changes were measured as seen in Figure 3.9.

A typical step input is shown in the inset. The chamber was filled with TE buffer to simulate a typical sample. When the input current reached steady state heating rates were approximately 30$^\circ$C/min, or at least 30x faster than typical heating rates on commercial MCA systems. The time taken by the chamber to attain 63% of the difference between its final and initial temperatures was observed to be 18 – 20 s during the heating cycle which is in close agreement with the theoretically calculated value of 13 s as seen in 3.1.1. Cooling response times were observed to be 20 - 22 s.

3.4.3 Demonstration of DNA melting

Four concentrations of dsDNA (742, 10, 1 and 0.1 $\mu$M) were used to demonstrate DNA melting within the device. Each test involved heating a chamber filled with dsDNA while recording its fluorescence intensity. The recorded fluorescence intensity values were then normalized and plotted (Figure 3.10). The corresponding negative first derivative graph of the melting curve shows a sharp peak at the dissociation point of 51.4$^\circ$C. In all the experiments, EvaGreen fluorescence intensity remained high until the dsDNA began to melt. The RTD resistance measurements recorded simultaneously during each fluorescence experiment were used for mapping the temperatures in the melting curves. The heater was excited with a step input current of 13 mA and heated for 180 s. At the end of the heating cycle the chamber reached an internal temperature of 77.7$^\circ$C. This ensured that all the DNA denatured completely. The decrease in fluorescence intensity for all samples began at the onset of heater turn ON and continued to decrease up to 77.7$^\circ$C with a melting peak at 51.4$^\circ$C.

Thermal lag

The experimentally obtained $T_m$ of 51.4$^\circ$C was greater than the supplier reported value of 45$^\circ$C. We attribute the variance of the RTD’s actual temperature measurement to the thermal mass contributions made by the various device components thermally bonded to the heat source. A temperature correction factor based on the thermal masses was applied to the temperature mapping provided by the calibration curve (as seen in section 3.4.1) to account for the variance. The heat transferred from the heater to the RTD through the substrate occurs laterally and is faster as it encounters less thermal resistance in addition to the lower thermal mass when compared to the thermal mass of the PDMS microfluidics and water sample.

Dye:DNA volumetric relationship

The fluorescence intensities for higher DNA concentrations 742 $\mu$M and 10 $\mu$M did not regain the fluorescence signal equal to that of the 1 $\mu$M and 0.1 $\mu$M concentrations. The lab protocol stated
Figure 3.10: The onset of dsDNA melting was detected at approximately 51°C and the melting peak appeared at approximately 51.4°C for all four dsDNA concentrations.

by the manufacturer for EvaGreen binding procedure recommended making 1:4 mixtures of dye:DNA volumes regardless of DNA concentrations. We theorize that the concentration of the
DNA does not follow a linear relationship to its corresponding EvaGreen fluorescence intensity as previously observed by Wang et al.[285].

3.5 Discussion

3.5.1 Eliminating bubble formation

We devised a simple step-wise approach to successfully eliminate bubble formation. Samples were first degassed for 20 min before they were injected into the chip, via a syringe pump, for experiments. The outlet tubing was pinched closed and the syringe pump was used to inject an additional 17 µL of buffer into the device, effectively pressurizing the chamber by 0.1 atm. Current was used to heat the sample to 95°C to cause bubble formation within the chamber. When the device was operated without pressurizing the chamber, bubbles formed within the first 10 s of heating. Figure 3.11(a) shows bubble formation at 30 s at a chamber temperature of 100°C. In contrast, pressurizing the chamber prevented bubble formation after 180 s at 95°C (Figure 3.11(b)).

![Figure 3.11: Bubbles rapidly formed in unpressurised chambers (a), but not in chambers that had been pressurized by 0.1 atm (b).](image)

3.5.2 Nanoliter DNA injection volumes

A 20-mer dsDNA sample (Integrated DNA Technologies, Iowa, USA) with the sequence 5′ - CTTGGGTGGAGAGGCTATTC -3′ was used to acquire melting curves. The supplier reported
Figure 3.12: An observed increase in intensity revealed a DNA plume that was automatically injected into the chamber during cooling. As the main chamber was cooled, the fluid volume decreased causing cooled solution from the inlet channel to enter the chamber, which was still above melting temperature.

The melting temperature was 55°C. Nuclease-free 1× TE buffer (30 mM Hepes, pH 7.5, 100 mM potassium acetate) was used for initial resuspension of the anhydrous DNA pellet to make a 500 µM stock solution. EvaGreen dye (Biotium Inc., California, USA) was used for the optical detection of DNA melting. One image was captured every three seconds. During the cooling cycle, after the dsDNA had been denatured, we observed dsDNA being injected from the channels. Figure 3.12 shows the sequence of events during a representative heating/cooling cycle. The intensity of a circular area, close to the inlet channel of the chamber, was measured to quantify the injection of unmelted dsDNA. The graph shows that while the heater is on, intensity decreases because of dsDNA melting within the chamber. As soon as the heater is turned off, cooling begins. Before the chamber cools and regains its original fluorescence intensity, a plume of unmelted dsDNA is introduced through the inlet channel. Plumes were observed during the cooling cycle of every experiment.
We attribute these plumes to the volumetric expansion of buffer during heating. The volume of the sample chamber is \( \approx 1 \) µL. Assuming a thermal expansion coefficient similar to water \((\approx 200^\circ C^{-1})\), the volume of the buffer in the chamber increases by 12 nL during heating, which is enough to push fluid down the inlet channel by about 1 mm. Fluid that has been expelled from the chamber into the inlet channel cools and the dsDNA anneals. As the fluid in the chamber cools and begins to contract, the fluid in the inlet channel enters back into the chamber below the melting temperature and fluoresces. This DNA injection strategy could be harnessed in future versions of the device to create minuscule volumes, on the order of nanoliters, for analysis.

### 3.6 Conclusions

DNA melting curve analysis can be performed successfully within a microfluidic reaction chamber without bubble formation and with sample volumes on the order of microliters. DNA samples at clinically relevant concentrations were melted and optically detected by the device.

**Note**

This chapter is being used in the preparation for the paper titled "Bubble-free Microenvironments for Glass-PDMS Hybrid Bioreactors" in the Journal of Micromechanics and Microengineering.
Chapter 4

Generation II: Wheatstone Bridge Based Glass-PDMS Hybrid

The sensitivity of the RTD was enhanced by incorporating a wheatstone bridge in conjunction with other electronic circuit components integrated on a make-shift breadboard. The wheatstone bridge circuit was printed photolithographically on a 100 µm thick micro coverslip and connected to the capture electronics for filtering out the melting signal from the overall device response. The design idea was to incorporate liquid samples to run melting experiments without the use of optics. A few design drawbacks including issues such as RTD self-heating are discussed.

4.1 Wheatstone Bridge Circuitry

The wheatstone bridge technique of measuring resistance changes was chosen over using instruments (such as the digital multimeter or DMM) for making direct measurements. The wheatstone bridge is a comparison technique which allows more precision than an independent measurement. The basis of the comparison technique involves assigning the baseline resistance as the reference point and measuring the change in resistance due to an external stimulus rather than measuring the total resistance. For example the wheatstone bridge technique determines that there is a 0.001% change in resistance of the sensor with reference to its own baseline resistance while a direct measurement with an instrument will not offer the same precision.

Resistive elements are the most commonly used sensors for detecting basic parameters such as temperature, strain and light [144, 68]. These resistive elements are relatively easy to fabricate and interface with signal conditioning electronic circuits [133]. Resistive sensors perform their sensing function by producing small percentage changes from their base value when subject to external stimuli from variables such as temperature change. When such resistive elements are
arranged in a ‘bridge’ configuration as seen in Figure 4.1, they are referred to as wheatstone bride circuits.

4.1.1 Background and applications

Wheatstone bridge circuits were first described by Samuel Hunter Christie, a British mathematician in 1833. However a British physicist Sir Charles Wheatstone popularized it as the ‘Differential resistance measure’ in 1840. He improved the design by including a variable resistor and discovered several new uses for it. Thus the circuit came to bear the name ‘Wheatstone’. Wheatstone realized that the bridge could be used to measure an unknown resistance by using the ratios of the three other known resistors. Wheatstone used the bridge for making soil comparisons [77].

This chapter introduces the concept of the wheatstone bridge and its application as a temperature sensor. The bridge was used to measure small resistance changes within the bridge structure.

4.2 Theory and Operation

A typical wheatstone bridge has four resistors that are connected in the form of a quadrilateral with each of the two arms of the circuit extending out to form a voltage divider as seen in Figure 4.1.
4.2.1 Modes of operation

Null

The bridge also has an excitation source connected across one of the diagonals and a voltage detector between the outputs of two voltage dividers. The condition of zero voltage at the output $V_{\text{out}}$ will occur when the resistance ratios between the series resistors on the left and right branches of the bridge become equal; a voltage is developed when there is a potential difference between them. The bridge is said to operate in null mode when $V_{\text{out}} = 0$ V. Regardless of the excitation type (AC or DC) or the magnitude of excitation the value of $V_{\text{out}}$ is forced to 0 V when the ratios of the resistances across the two voltage dividers become equal as shown in the equation below

$$\frac{R_2}{R_1} = \frac{R_4}{R_3} \quad (4.1)$$

Commercially available wheatstone sensors are designed such that the ratio of $R_4/R_3$ is a known constant since it is impossible to manufacture a balanced wheatstone bridge with passive elements. No two wires or solder connections can be made exactly identical, therefore a variable resistance is used in conjunction with fixed resistors [65]. If the unknown resistance were to be $R_2$ and $R_1$ is a variable resistance, then $R_1$ can be adjusted until a null value is seen at the output $V_{\text{out}}$. Typically, such null detector systems could be used as feedback in circuitry involving human and robotic elements. The feedback mechanism forces the sensing resistor to balance the bridge in order to provide a null output by adjusting the measured parameter.

Detector

Most sensor applications which employ bridges rely on the deflection of one or more resistors from its initial set point. The measured deviation from this set point or base value is marked as an indication (directly or indirectly) of the magnitude in the parameter measured. In this research, we have measured a change in the output voltage $V_{\text{out}}$ as an indicator to temperature change that the resistive sensor (RTD) detects. The output voltage $V_{\text{out}}$ can be calculated theoretically as shown in the equation below

$$V_{\text{out}} = \frac{R_2}{R_2 + R_1} V_{\text{in}} - \frac{R_4}{R_4 + R_3} V_{\text{in}} \quad (4.2)$$
Figure 4.2: Non-linear wheatstone bridge output voltage: A bridge circuit schematic showing line resistors of small resistance values with a 1% mismatch in the resistance ratios between the two arms of the bridge. The graph on the right represents the corresponding output response to its incremental resistance ratio change.

4.2.2 Linearizing wheatstone bridge output

In this chapter, we discuss the methodology of patterning resistive sensors on glass to amplify and capture a high signal-noise ratio response from the bridge by incorporating signal conditioning electronics. While an independent resistor may increase linearly with temperature as illustrated in section 3.4.1 the output of a wheatstone bridge comprising such resistors may exhibit non-linearity. Non-linear bridge responses can be attributed to two main reasons. While one reason is the inherent ‘unbalanced’ state of the bridge, another is the response quality of the active element such as an RTD [137, 191].

The unbalanced wheatstone bridge

Small resistance mismatches between the left and right arms in the bridge can result in non-linear output voltage. For instance if the ratio $R_2/R_1$ changes as a small percentage of the ratio $R_4/R_3$ the output voltage swings in a non-linear fashion as seen in the Figure 4.2. Commercial sensor manufacturers employ laser-trimmed resistors to minimize resistance mismatches. Non-commercial microfabrication invariably resulted in mismatched resistance values due to human error and side-effects of batch processing. In other words though the resistor pairs $R_2, R_4$ and $R_1, R_3$ were designed to be identical a resistor in each pair varied from the other by an average of $5 – 10\%$. We addressed the resistance mismatch problem by patterning simple line resistors with small resistance values to ratiometrically balance their larger coil resistance counterparts as seen in the bridge circuit schematic in Figure 4.2. Line resistors are less-prone to mismatches due to their simplicity and exhibited resistance variance of $\approx 1\%$. 

57
Now if the bridge with mismatched RTDs as seen in Figure 4.2 had its sensing RTD \( R_3 \) subjected to an external stimulus causing its resistance to experience a momentary 25% decrease the bridge output calculated from the equation 4.2 would show a ‘break’ in the quadratic trend as observed in Figure 4.3. This mechanism of sensing using a wheatstone bridge was employed in designing generation II devices.

**RTD non-linearity**

RTD non-linearity can occur either due to the material properties of the RTD or inherent characteristic of the RTD such as high resistance swings while sensing and its polynomial response to temperature changes. We minimized the issue of non-linear output response due to the RTD characteristic by introducing operational amplifiers (Opamps) in the signal conditioning circuit as described in section 4.7. Opamps offer excellent DC characteristics and expand the possibilities of adding more electronic components for acquiring an enhanced signal with minimal noise.
4.3 Instrumentation Overview

4.3.1 Operational amplifiers

Operational amplifier (opamp) circuits consist of a network of elements such as transistors, capacitors and resistors. Typical opamps have two input terminals and one output terminal. In an ideal opamp the high impedance input terminals remain unaffected by signals common to both inputs (in other terms: common mode or CM)[268]. However, opamps demonstrate high gain when differential signals are applied at its inputs. Opamp gain is a dimensionless variable which is calculated by the following equation

\[
Gain = \frac{Output\ voltage}{Input\ voltage}
\] (4.3)

With laser trimmed resistor elements constituting a significant percentage of the internal circuitry, opamps provided accurate gain and high common-mode rejection. Commercially available integrated circuit (IC) opamps available in dual-in-line pin (DIP)[96] packaging were used which enabled easy surface mounting on solderless breadboards. A variety of opamps were integrated to form generation II circuitry and each was meant to perform a specific function. The following sections summarize the fundamental operational theories behind each type of opamp used.

4.3.2 Buffer amplifiers

The buffer opamp configuration renders it a high fidelity voltage follower by mirroring its input precisely. The advantages of using buffer amplifiers are multi-fold [137]. While the inherent high input impedance of the buffer opamp draws very little current from the input voltage terminal, the output is capable of sourcing a much larger current. This mechanism of voltage transformation allows the use of buffers to help string together sub-circuits in large circuits with several stages without impedance mismatch problems. A schematic of the buffer opamp is shown in Figure 4.4(a).

A unity gain buffer amplifier was created by shorting the negative input terminal to the output while the positive input terminal was interfaced with one of the bridge outputs. This negative feedback allowed the output voltage to maintain the voltage value very close to the input voltage value. The general purpose LM741 opamp (Texas Instruments, USA) was used in the circuit shown in Figure 4.7(a).
4.3.3 Differential amplifiers

An opamp without feedback automatically behaves as a differential amplifier by amplifying the voltage difference appearing on the two input terminals. The amplification factor can be controlled by using external resistors to control the gain on the differential amplifier. When four resistors are connected to a general purpose opamp in a voltage divider fashion as seen in Figure 4.4(b), such a circuit forms a basic differential opamp. When all the resistors are equal in value, the voltage gain is 1. However by setting the ratios $R_2/R_1$ and $R_4/R_3$ equal, the gain may be set by directly manipulating the ratio $R_4/R_3$. The output voltage $V_{out}$ will be directly proportional to the difference in input voltages ($V_1$ and $V_2$) and is governed by the equation

$$V_{out} = (V_2 - V_1) \frac{R_4}{R_3}$$

(4.4)

A schematic of a regular differential amplifier with manual gain control is shown in Figure (b). The INA106 (Texas Instruments, USA) precision gain differential amplifier with a set gain of 10 was used in the circuit shown in Figure 4.7(a).

4.3.4 Instrumentation amplifiers

The most basic model of an instrumentation amplifier consists of at least three typical opamps. It is a combination of buffer and differential amplifiers. It has a pair of differential inputs that are buffered using two opamps. Both input impedances are high on the order of $10^9 \, \Omega$ and balanced. The gain can be controlled by the use of one external resistor. The output voltage of an instrumentation amplifier is referenced to a specific value depending on the application. The output then amplifies the micro-volt level differential signals in accordance with the reference point. A schematic of a typical instrumentation amplifier is shown in Figure 4.4(c).

A low power instrumentation amplifier INA128 (Texas Instruments, USA) was used in the final stage of the signal conditioning circuit as seen in 4.7(a). The INA128 offered an adjustable gain ranging between 1 to 10,000.

4.4 Materials and Methods

4.4.1 Experimental setup

A block diagram of the experimental setup as seen in the Figure 4.5 shows the three major components: wheatstone bridge device, signal conditioning circuit and the data acquisition unit. The wheatstone bridge was powered by an Agilent dual terminal DC power source (Agilent
Figure 4.4: Types of opamp circuits: (a) Buffer amplifier: The gain = 1 since output $V_{out}$ follows input $V_{in}$ (b) Differential amplifier: Adjusting the value of $R_4/R_3$ sets the value of gain (c) Instrumentation amplifier: External resistor $R_{gain}$ sets the value of gain.

Figure 4.5: Block diagram of experimental setup showing the interfacing of the wheatstone bridge device with the modular capture electronics. The DC input $V_{in}$ from the voltage source was converted to constant current mode for exciting the bridge.

34410A, Agilent Technologies, USA). The differential output voltage of the wheatstone bridge was connected to the signal conditioning circuitry. An analog to digital converter or A/D system was used to discretize the incoming analog input. Otherwise called as data acquisition system it sampled the analog signals periodically and converted them to digital signals comparable to the analog input’s voltage amplitude. The data acquisition system (NI DAQ 6009, National Instruments, USA) sampled the conditioned signal at 1 kHz and relayed to a computer for analysis.

4.4.2 Device fabrication

Fabrication methods described in 3.3.4 were employed to pattern a typical wheatstone bridge consisting of four resistors on a 100 µm thick glass coverslip. The large resistors were designed to
Figure 4.6: Optical micrograph of device (top view) showing patterned nickel resistors in a wheatstone bridge configuration occupying a circular footprint <1 mm in diameter. The larger polygonal and circular parts were designed as probe pads for interfacing electronics have $\approx 150$ $\Omega$ base resistance while the smaller line resistors were designed to have one-tenth the value of their larger counterparts. The lower resistors were intentionally patterned as simple line resistors with smaller resistance values to avoid fabrication errors which could lead to non-linear output voltage. An optical micrograph of the device is shown in Figure 4.6.

4.4.3 Circuit components

The signal conditioning unit was a network of opamps as seen in Figure 4.7. It consisted of buffer amplifiers connected to each of the bridge output terminals. The buffered signals were fed into the first stage of amplification through a differential opamp (indicated as ‘Diff Amp 1’) with a gain of 10. The output of this differential opamp was then fed into a similar differential opamp which provided further amplification by a factor of 10. The second stage differential opamp (indicated as ‘Diff Amp 2’) had a programmed DC offset as a comparative input for trimming the response further. The final signal conditioning stage consisted of an instrumentation amplifier (indicated as ‘Instr Amp’) with variable gain which compared the differential output with the DC offset. The low impedance output data produced by the instrumentation amplifier was fed to the data acquisition board (indicated as ‘DAQ’) for dynamically relaying the final response to the computer. All opamp ICs described above were integrated as a modular signal conditioning unit using a solderless prototyping breadboard (Digikey, USA) and jumper wires.
4.4.4 Operation methodology

The wheatstone bridge network was excited by giving a ramp input in the constant current mode. Labview was used to control the Agilent DC power supply to output a programmed set of discrete voltage values that translated into the excitation current to the bridge. This excitation current which increased in a ramp-like fashion caused the resistors in the bridge to increase in resistance and hence rise in temperature. The resistors acted as on-chip heaters by creating localized heating areas. The bridge resistors also performed the function of temperature sensing by detecting the difference in heating rates for various types of melting events. Therefore the bridge output would account for both the heating and temperature sensing functions.

In the signal conditioning circuit, the DC offset required at the input of differential amplifier 2 in the signal conditioning circuit was pre-calculated and fine-tuned before the start of the experiments. Fine-tuning the DC offset values involved mathematical calculations and experimental trial-and-error to obtain the closest possible voltage values to reject common mode signals and trim the response of the bridge output. The amplified output was collected as a voltage response which was then digitally sampled by the data acquisition system.

4.5 Results

4.5.1 Bridge response: control

An empty bridge device was used for obtaining the base response as seen in Figure 4.8. The inset shows the characteristics of the bridge input excitation current which had a linear increase from 0 – 18 mA. The input current raised the temperature of the bridge resistors from 23°C...
Figure 4.8: Wheatstone bridge control response: The excitation current shown in the inset delivered current to increase temperature at the rate $\approx 11^\circ\text{C}/\text{min}$. The signal conditioned output recorded by the instrumentation amplifier maintained $\approx 0$ V value which indicated that all resistive elements experienced identical heating rates.

to $\approx 61^\circ\text{C}$. The corresponding bridge outputs ($V_{\text{out}}^+$ and $V_{\text{out}}^-$) followed each other closely with minimal deviation from each other. The DC offset applied at the second differential amplifier delivered a voltage ramp signal to trim out the common mode signals. The instrumentation amplifier output which ultimately captured the trimmed and amplified bridge response was plotted on the same graph for comparison. Its output remained close to 0 V throughout the heating cycle indicating that there was no change in heating rate as all the resistors in the bridge were exposed to the same sample i.e. air. The output of the instrumentation amplifier suddenly rises to a stable positive voltage value when the current input is turned OFF instead of reverting to its baseline value as seen in Figure 4.8. This anomaly occurs as the DC offset input to the instrumentation amplifier was provided by a manually controlled DC power unit. The unmanned DC offset input which was no longer required after the current was turned OFF was captured by the instrumentation amplifier and multiplied with the appropriate gain. This anomaly did not impact the experiments as it occurred after all the relevant data was collected.
4.5.2 Bridge response: Wax melting

For proof-of-concept a piece of wax with an average mass of 800 µg was placed on the sensing resistor $R_3$ while the remaining three resistors were left blank. The excitation current when ramped from 0 – 18 mA heated the wax sample. The output response of the bridge showed a sharp peak at $\approx 51^\circ$C as seen in Figure 4.9. The temperature profile was obtained from a calibration curve similar to 3.4.1 where the bridge device was exposed to equilibrium temperatures inside a conventional oven.

![Figure 4.9: Melting response of wax: The bridge outputs $V_{out}^+$ and $V_{out}^-$ showed signs of cooling 30 s after the excitation current was turned OFF. The peaks in the melting response were indicative of a phase change event. The consecutive peaks after the first melting peak at $\approx 51^\circ$C resulted from the uneven melting and the irregular flow pattern of the liquefied wax sample on the bridge sensor. The energy required to melt the 800 µg piece of wax was calculated to be 168 mJ.](image)
4.6 Discussion

4.6.1 Thermal profile of wax

The heat of fusion ($\Delta H_{\text{fusion}}$) of paraffin wax was 220 J/g [200]. The heat capacity or the amount of energy ($Q$) required to melt a specific mass ($m$) of the specimen is given by the thermodynamic equation

$$Q = m \Delta H_{\text{fusion}}$$  \hfill (4.5)

$Q$ was calculated to be 176 mJ. The peaks that occurred during the heating cycle were a result of a phase change event that changed the wax from a solid to liquid. During phase change the amount of energy ($Q$) delivered to the wax continued to increase while the temperature remained constant. This subtle temperature discrepancy on the sample holding resistor $R_3$ momentarily changed the $R_4/R_3$ ratio. The wheatstone bridge was knocked out of balance during this phase change instance because the $R_2/R_1$ ratio continued changing unaffected by the wax while the temperature of the counter-balancing resistance ratio $R_4/R_3$ changed dramatically around the melting peak.

The corresponding temperature change detected by the bridge was calculated by using equation (3.4). Assuming the current during the time of melting was between 13 – 14 mA the circuit detected a resistance change of 192 mΩ during the melting. This $\Delta R$ value was obtained from Ohm’s law as described by the equation below (4.6)

$$\Delta R = \Delta V/I = 90.37 \text{ m}\Omega$$  \hfill (4.6)

where $\Delta V$ was the difference in the output voltage $V_{\text{out}}$ value directly from the bridge without considering the gain of 200 from the opamps. $\Delta V$ obtained from Figure 4.10 was 1.22 mV. Substituting the above values in equation (3.4) with $R_i$ as 150 $\Omega$ the temperature difference $\Delta T$ detected by the bridge circuit was calculated to be 186 m°C.

4.6.2 Liquid samples

PDMS chambers with two 1 $\mu$L volume reaction chambers were created using soft lithography principles described in section 3.5. These reaction chambers when aligned with the two larger resistors $R_1$ and $R_3$ and irreversibly plasma bonded to the glass side of the device. These formed the liquid sample reservoirs with access ports to interface with an external syringe pump.
Design idea

Samples of equal volume and composition (such as buffer solution) would theoretically have the same heating rates and hence the bridge output would show no signs of differential heating similar to heating air in Figure 4.8. On the contrary if one of the chambers had a substance capable of melting suspended in buffer, while the other had only buffer the differential signal obtained from heating these disparate substances would record the melting phenomenon.

Related limitations

Following the principle above, a control experiment containing two equal volumes of 1×TE buffer were pressurized and heated. The bridge outputs from different devices showed little repeatability. We attribute this lack of repeatability to the PDMS chamber lying directly on the smaller line resistors in the bridge. The presence of the PDMS not only added thermal mass to the device but differences in alignment with every device also gave rise to the disparity in the control runs. The addition of the thermal mass from the PDMS reduced the sensitivity of the device to detect thermal energy changes less than 186 mJ much like the thermal changes associated with DNA. In addition we suspected that sensitivity of the wheatstone bridge was compromised due to its use as both the heating and sensing element. Commercial RTDs limit their excitation current to 1 mA to avoid self-heating. However in the bridge device the currents used to raise the temperature to $\approx 61^\circ$C were as high as 18 mA which invariably led to RTD self-heating.

Chapter 5 describes the steps taken to circumvent the design drawbacks mentioned above.
Chapter 5

Generation III: Film Device for Droplet-based Detection

Key microfabrication steps in the silicon process flow were redesigned to include a 12.5 \(\mu\)m thick polyimide (PI) flexible film as the substrate for patterning the nickel electrodes in lieu of the glass micro coverslip. The sample was applied as a 0.5 \(\mu\)L droplet encapsulated by an oil drop to avoid evaporation. Thus the overall thermal capacitance of the system was minimized by reducing the sample volume, eliminating PDMS microfluidics and reducing the substrate thickness by 10× times when compared to the predecessor glass-PDMS device. The self-heating RTD problem was completely eliminated by employing a separate heater and exciting the RTD wheatstone bridge sensor using AC voltage. Custom electronics incorporated modular analog chip circuitry including a MODEM (modulator-demodulator) chip with built-in lock-in amplification capability to increase the overall signal-to-noise ratio.

5.1 Electronic Instrumentation Overview

5.1.1 Wheatstone bridge: AC excitation

DC excitation is the most common method of powering a wheatstone bridge (as explained in section 4.4.1). DC methods are widely used due to their simplicity and cost-effectiveness but suffer drawbacks such as inheriting low frequency 60 Hz noise from the power supply [145] and signal drifts caused due to DC offset errors [137]. Exciting the bridge with alternating current (AC) has specific advantages over DC excitation. AC excitation allows the signal to be amplified, level-shifted and filtered to acquire narrow pass bands and will be independent of all DC offsets originating from the circuit components [204]. The alternating nature of the AC excitation allows the cancellation of the circuit’s offset errors during each cycle when the polarity reverses,
resulting in a net bridge output that is free of the offset error term. Measurements can also be made faster with AC bridges as they stabilize quickly [9] and do not require circuit warm-up before recording the first set of readings [89] unlike DC bridges. Readout from AC bridges have a much lower noise floor than that from DC bridges because they operate above the corner frequency $1/f$.

Typical AC bridges comprise impedance elements ($Z$) connected in the form of a quadrilateral as seen in Figure 5.1. Impedance elements can include capacitors and inductors. Consequently

![AC Wheatstone Bridge Schematic](image)

**Figure 5.1**: Schematic: Typical AC wheatstone bridge. The alternating input can be sine, square, triangular or saw-tooth waveforms of a particular amplitude and frequency.

the bridge balance conditions include not only matching the magnitudes of the impedances but also their phases $\phi$. The two equations (5.1) and (5.2) for magnitude and phase respectively must be satisfied simultaneously to balance the AC bridge.
The output of the bridge $V_{\text{out}}$ is also alternating in nature. In this thesis the impedances ($Z$) are replaced by resistances ($R$) as there were no active capacitive and inductive elements included in the circuit. The arms of the bridge which include resistors are generally not affected by stray capacitances formed in ground loops and have been neglected for precision thermometry studies.

### AC operation of wheatstone bridge

In the AC mode of operation a low frequency signal typically ranging from $(1 - 1000 \text{ Hz})$ excites the bridge. The bridge balance conditions are achieved by tuning a variable resistor or reactance element (capacitor or inductor) both for magnitude and phase in order to obtain a null reading. The voltage output of the bridge is fed through an amplifier working above the $1/f$ frequency cut-off region. The amplified AC output is converted to a DC signal proportional to the small change in resistance by employing a phase-sensitive rectifier followed by narrow band filters.

#### 5.1.2 Lock-in amplifier

Lock-in amplifiers are phase-sensitive detectors that are capable of sensing nanovolt signals \[36\]. Figure 5.2 is a schematic of the working principle of the lock-in amplifier. The advantage of using lock-in amplifiers is the noise immunity they offer for detecting a single component embedded within a noisy signal at a specified frequency and phase \[189\]. Other signals which get intricately incorporated within the output signal get rejected by lock-in amplifiers as they are not synchronized with the frequency and phase specifications of the desired signal. Lock-in amplifiers require an input signal along with a reference. A noisy output signal from an experiment along with an externally controlled reference signal $R_{\text{ext}}$ form the inputs to a lock-in amplifier. $R_{\text{ext}}$ can be sourced from a simple function generator. Sophisticated lock-in amplifiers generate their own stable internal oscillator $R_{\text{int}}$ which is synchronized with $R_{\text{ext}}$ both in frequency and phase. The phase-locked loop (PLL) generates a DC signal after integrating over a certain time interval to reject all other signals not synchronized with $R_{\text{ext}}$ \[8\].

\[
Z_2 Z_3 = Z_1 Z_4 \tag{5.1}
\]

\[
\phi_2 + \phi_3 = \phi_1 + \phi_4 \tag{5.2}
\]
Figure 5.2: Lock-in amplifier: Working principle. A phase-locked loop is created when the lock-in amplifier generates its own internal reference signal $R_{\text{int}}$.

### 5.1.3 RC filters

Resistor-capacitor (RC) filters are circuits composing passive elements such as resistors and capacitors which operate without an internal gain unlike active elements such as transistors or opamps. A simple low-pass (LP) filter is constructed by connecting a resistor ($R$) in series with a capacitor. The first-order output $V_{\text{out}}$ is measured across the capacitor ($C$). A LP filter rejects high frequency signals and passes low frequency signals set by the cut-off or corner frequency $f_c$. Figure 5.3 (a) shows the basic construction of an LP filter. Unlike the resistance of $R$ the reactance of $C$ ($X_C$) is much larger at low frequencies as it is inversely proportional to frequency as seen in equation (5.3). The corner frequency $f_c$ for a LP filter can be designed by selecting appropriate $R$ and $C$ values. The output $V_{\text{out}}$ varies inversely with the total impedance of the circuit ($Z$) as seen in the equation. The impedance $Z$ is defined as $\sqrt{R^2 + X_C^2}$.

\[
X_C = \frac{1}{2\pi fC}, \quad f_c = \frac{1}{2\pi RC}, \quad V_{\text{out}} = V_{\text{in}} \frac{X_C}{Z}
\]  (5.3)
Figure 5.3: First order low-pass filter (a) Circuit schematic containing one reactive component $C$ (b) Frequency response: The response above the corner frequency $f_c$ gets attenuated rapidly at the rate of 20 dB/decade.

5.2 Background: Materials

5.2.1 Ferric chloride etching of copper

Most ferric chloride ($\text{FeCl}_3$) etchant preparations etch copper and nickel at similar etch rates. $\text{FeCl}_3$ ionizes in water to form ferric and chloride ions. The ferric ions combine with hydroxide ions to form ferric hydroxide ($\text{Fe(OH)}_3$ or rust) which can be observed as a brown solid precipitating in the solution. This process leaves an excess of hydrogen ions which react with copper. Copper gets etched vigorously without any gaseous emissions.

A summary of the basic steps describing the chemistry of the etching reaction is shown in equation (5.4) where (a) describes ferric chloride ($\text{FeCl}_3$) oxidizing metallic copper ($\text{Cu}$) to cuprous chloride (CuCl) and ferrous chloride (FeCl$_2$) and (b) describes the reaction of the newly formed CuCl with the active etching ingredient FeCl$_3$ [69] to form cupric chloride (CuCl$_2$). Both FeCl$_3$ and acidic CuCl$_2$ simultaneously etch copper. The solution is treated as spent when the bath turns green/blue indicating that CuCl$_2$ precipitated from the solution as a result of exceeding the solubility limit of copper.
FeCl₃ + Cu = FeCl₂ + CuCl \quad (5.4a)

FeCl₃ + CuCl = FeCl₂ + CuCl₂ \quad (5.4b)

5.2.2 Polytetrafluoroethylene

Polytetrafluoroethylene (PTFE) or Teflon (C₂F₄)ₙ is a fluoropolymer and its basic structure composes two carbon atoms surrounded by an outer shell of four fluorine atoms which form long chains to constitute the polymer molecule. Generally surface atoms are less strongly bound and hence possess higher energy than their counterparts in the bulk material. Surface free energy of a solid is defined as the sum of all the excess surface atom energies [11]. Research teams have established that the surface free energy of teflon is very low [33, 128] by conducting contact angle measurements [44] and applying corresponding mathematical calculations as established by Wu et al. [294].

5.2.3 Glycerin

Generally glycerol or glycerin is used to prevent the freezing of storage buffers at −20°C. In addition glycerol is a popular choice for creating non-aqueous DNA solutions [165, 213, 46]. Glycerol is denser than water but is half as thermally conductive as water. UV absorbance studies of DNA suspended in 99% glycerol have demonstrated that glycerol supports both thermally induced denaturation and consequent renaturation [26]. The reported $T_m$ value of the model DNA suspended in glycerol was $\approx 30°C$ lower than when suspended in aqueous solvents. Similarly the egg-white protein lysozyme exhibits [142] favorable structural integrity when suspended in glycerol. PCR with glycerin-diluted DNA samples was reported to yield higher amplification rates after multiple rounds of repeated freezing and thawing than when dissolved in water [245].

In this project glycerol or glycerin (99% pure, laboratory grade) was used to prepare DNA stock solutions due to two distinct advantages. Firstly glycerol provided a stable non-aqueous environment for favorable thermal denaturation of DNA. Secondly glycerol not only exhibits half the specific heat of water but also does not evaporate when heated.

5.2.4 Salmon DNA

Sperm cells collected from the testes of salmon is a good source of non-mammalian dsDNA. Many methods describe the process of extraction of the dsDNA [185, 231]. It composes 2000 base pairs [264] with $\approx 41\%$ G-C content. Several research groups have reported calorimetrically
obtained melting temperatures ranging from 69 − 88°C [14, 107, 180]. The wide range in the $T_m$ values was contingent on the purification protocol and the salt concentration in the suspension solvent. In this project commercially available salmon sperm DNA was used after creating a suspension in 1× TE solution and tested without employing purification protocols or shearing.

5.3 Materials and Methods

5.3.1 Experimental setup

A block diagram of the experimental setup is as shown in Figure 5.4. The sensing RTD $R_2$ of the PI film device was incorporated in the wheatstone bridge. The bridge was excited with AC voltage using an external function generator. The output of the bridge was sent into the signal conditioning electronics for rejection of unwanted signals, amplification of the desired signal and filtering before relaying the measurements to the data acquisition board (DAQ) via a universal serial bus (USB). The heater was heated using a computer controlled DC power source via a general purpose interface bus (GPIB). Custom Labview programs were used to control 2-way communications between the DC supply and the DAQ. The wheatstone bridge along with the instrumentation amplifier (depicted as Instr Amp), lock-in amplifier (depicted as MODEM) and 3-pole low-pass filter were constructed on a prototyping board. The RTD of the PI film device depicted in the block diagram as $R_2$ was connected to the bridge using...
test leads as shown in Figure 5.5. The two resistors $R_1$ and $R_3$ were 2-wire resistors with fixed resistances. A potentiostat with variable resistance ($0 - 100 \, \Omega$) was used for balancing the bridge. An arbitrary function generator (Agilent 33220A, Agilent Technologies, USA) was used to excite the bridge with a square waveform of peak voltage $V_{pp} = 1 \, V$, frequency 400 Hz and 50% duty cycle. The same signal was used as the reference signal for the lock-in amplifier. The resistance change measurements made by the bridge were amplified by an IC instrumentation amplifier with variable gain ($1 - 1000$) adjustable by an external resistor $R_g$. Each pole in the 3-pole LP filter contained an RC filter circuit with an $f_c = 16 \, \text{mHz}$. The filtered output was relayed using a parallel bus interface which used IEEE-488 standards of short range digital communications. Specifically a general purpose interface bus (GPIB, Keithley Instruments, USA) was used to communicate with an A/D converter (NI DAQ 6225, National Instruments, USA) which was in turn connected to a computer for data storage and analysis. The sampling rate of the A/D converter was 1 kHz. A custom acrylic contraption was designed for mounting the flexible substrate device. Techniques described in section 3.3.1 were used to machine the jig and to interface the device with the real-world instruments. A photograph of the device mounted on the jig is seen in Figure 5.6.
5.3.2 Buffers and reagents

Salmon sperm DNA (D1626, Sigma Aldrich, USA) of molecular weight 1.3e6 g/mol was used to make a stock solution of 100 µM by suspending 13 mg of the DNA in 100 µL of 1× TE buffer (IDT, USA). Three DNA concentrations (10, 1 and 0.1 µM) were prepared by serial dilution of the stock solution. The stock solution was prepared by vortexing the thread-like lyophilized DNA until a viscous, optically opaque homogenous solution was obtained in a scintillation vial. The contents were then transferred to an eppendorf tube for centrifugation. Centrifugation for ≈ 1–2 min removed all the trapped air bubbles in the solution. A similar bubble-removal method was applied to the serial dilutions. All eppendorf tubes were momentarily vortexed just before pipetting each sample for droplet deposition to avoid sedimentation.

Commercially available candle-maker’s paraffin wax was purchased. Some wax was melted in a beaker and cooled. Blunt-tipped syringe needles of varying inner diameters were employed to core out wax cylinders from the beaker. These cylinders were cut as discs of varying thicknesses to create wax samples for melting experiments with a known average mass.

5.3.3 Device fabrication

A schematic representation of the process flow is shown in Figure 5.7. Samples of 12.5 µm thick copper-coated polyimide (PI) sheets were obtained from Dupont (Pyralux copper clad laminates, Dupont, USA). The 9 µm copper film supported the 12.5 µm PI film. The copper-clad PI films were cut into 3” diameter circular discs and mounted on 3” silicon wafers (<
Figure 5.7: Process flow for microelectrode fabrication on polyimide flexible film. (1) Clean copper-clad PI substrate. (2) Spin coat 1.5 μm thick photoresist on surface. (3) Soft bake at 95°C. (4) Expose to UV light through a photomask to yield underexposed portions in the photoresist. (6) Image reversal bake cross-links exposed photoresist pattern. (7) Flood expose without a mask to expose remaining photoresist. (8) The flood-exposed photoresist pattern is developed. (9) Nickel is evaporated on the substrate surface. (10) Remove photoresist using a solvent. (11) Spin coat photoresist to mask the nickel microelectrodes and introduce to a ferric chloride based copper etchant. (12) Remove photoresist using acetone and rinse experiment-ready device with IPA.

100 >, p-type, University wafers, USA) using carbon tape for providing mechanical support during the process flow. The PI side of the copper-clad film faced the user for patterning the microelectrodes. The discs were thoroughly cleaned with acetone and isopropanol (IPA) and rinsed with DI water. Excess moisture was driven out by heating the discs on a hotplate at 200°C for 10 min. Image reversal photoresist was used for patterning the microelectrodes. The copper-clad PI discs were coated with 1.5 μm of the image-reversal photoresist AZ 5214 E-IR (Capitol Scientific Inc., TX, USA) by spin-coating at 4000 rpm for 45 s using a Laurell spin coater (WS-400-6NPP, Laurell Technologies, PA, USA). The discs were then soft-baked at 95°C for 1 min to improve adhesion to the PI surface and to drive out excess solvent in the photoresist. The photoresist layer was patterned via a photomask through an i-line optical filter with a minimal exposure dose of 30 mJ/cm² to form trapezoidal exposed areas within...
the photoresist thickness. An image reversal bake step was performed after the exposure on a 115°C hot plate for 30 s which rendered the exposed areas cross-linked to form a developer insoluble and light-insensitive substance while the unexposed areas remained chemically similar to normal photoresist. A flood exposure of 600 mJ/cm² following the reversal bake step ensured previously unexposed areas became completely soluble. The substrate was then developed in Microposit metal ion-free CD-26 developer (Rohm and Haas Electronic Materials LLC, MA, USA) for 25 s, leaving a negative image of the mask pattern with retrograde sidewall profiles to facilitate good lift-off processing. An electron beam evaporator was used to deposit a 200 nm thick film of nickel (Kurt Lesker, 99.99% pure) on the patterned PI discs. The nickel-coated substrates were then treated with acetone to lift off nickel from the unpatterned areas, rinsed with de-ionised water, and dried with nitrogen.

The following steps were performed to etch the copper from the PI film. A positive photoresist S1813 (Shipley 1813, Micrchem, USA) was spin-coated on the nickel microelectrodes and hard-baked at 115°C for 1 min. This ensured that the nickel electrodes were protected against etching by the copper etchant during accidental contact. The discs were immersed in a bath of copper-etchant (APS-100, Transene, USA) with continuous agitation enabled by a magnetic road. The etch rate of copper was \( \approx 80 \text{ Å/sec} \).

**Teflon coating**

Teflon resin pre-dissolved 1:100 (w/w) in solvent Fluorinert FC40 (Teflon AF - 1600, Dupont, Canada) was spin-coated at 2000 rpm for 1 min. The teflon film was then cured on a hotplate at 160°C for 10 min to yield a final thickness of 50 nm [1]. The teflon was used to coat the PI film on the nickel-free side (Teflon coating step not shown in Figure 5.7) for creating a hydrophobic/oleophobic surface [218, 2]. Finally the discs were separated from the silicon wafers for testing. The photograph of the finished device is as seen in Figure 5.8.

**5.3.4 Droplet application**

The surface wettability of the PI film was dramatically reduced due to the low surface energy imparted by the teflon coating. This enabled both the sample droplet and the encapsulating oil drop to form a bead at > 90° contact angle with the surface. Droplets of two different compositions were applied. Figure 5.9a shows a 5 µL droplet of DNA mixed with glycerin while Figure 5.9b shows a 0.5 µL of the liquid sample encapsulated by oil. The liquid bead was placed on the RTD with a micropipette such that the diameter of the contact with the film covered only the diameter of the RTD. A 5 µL mineral oil drop was deposited on top of this sample bead such that it encapsulated it without air gaps. The diameter of the encapsulating oil droplet extended up to the diameter of the heater as seen in both droplet schemes depicted in Figure
The width of the nickel traces was 30 μm. The sensor was designed to have a nominal resistance of ≈ 100 Ω at room temperature, while the heater resistance was designed to be ≈ 200 Ω.

(a) Cross-section of hydrophobic 5 μL DNA mixed with glycerin drop placed on the device (b) 0.5 μL DNA sample encapsulated by a 5 μL mineral oil drop.

DNA droplets within oil encapsulations were tested on a 100 μm thick glass-based device.
Figure 5.10: Optical melting curves of dsDNA: The melting peak appeared at approximately 46°C for all four dsDNA concentrations. Total run time = 160 s and each curve is the average of 3 trials (n=3).

patterned with a heater-RTD similar in footprint as seen in Figure 5.8. The PI device was not used as the film was not optically transparent. Four concentrations of dsDNA (742, 10, 1 and 0.1 µM) were used to demonstrate DNA melting within the device. Buffers and reagents used were similar to those described in section 3.3.2. The heater was given a step input of 7 V and was left ON for 30 s to heat up to ~80°C. Each test involved heating a chamber filled with dsDNA while recording its fluorescence intensity. The recorded fluorescence intensity values were then normalized using the procedure described in section 3.3.3 and plotted in Figure 5.10. Each curve displayed in the graph was an average of three trials.

The corresponding negative first derivative graph of the melting curve shows a sharp peak at the dissociation point of 46°C. In all the experiments, EvaGreen fluorescence intensity remained high until the dsDNA began to melt. The RTD resistance measurements recorded simultaneously during each fluorescence experiment were used for mapping the temperatures in
the melting curves. The heater was excited with a step input current of 13 mA and heated for 180 s. At the end of the heating cycle the chamber reached an internal temperature of 80.2°C. This ensured that all the DNA denatured completely. The decrease in fluorescence intensity for all samples began at the onset of heater turn ON and continued to decrease up to 77.7°C with a melting peak at 46°C.

5.4.2 Demonstration of wax melting

Cylindrical wax pieces of mass ≈ 800 µg each were used in the melting experiments. The supplier stated melting temperature of wax $T_m$ was 55°C. Wax melting was tested both in glycerin and oil encapsulations and their melting characteristics were recorded by the DAQ operating at a sampling frequency of 1 kHz and the circuit at a gain of 1000.

Device parameters and temperature mapping

Table 5.1 describes device parameters while table 5.2 describes the RTD temperature mapping as shown below. The device described in the device parameters table was heated from room temperature to a maximum of 75°C. Every 1 V change in $V_{out}$ indicated a temperature change of ≈ 18.5 °C as seen in the temperature mapping table. Temperature mapping was performed by recording the $V_{out}$ voltage output from the DAQ and the corresponding increase in total RTD resistance value connected in series with a 100 Ω variable potentiostat. The value on the potentiostat was measured manually using a DMM to determine the change in total resistance value. The additional potentiostat value added to the base RTD value is depicted as ($\Delta$RTD) as seen in the temperature mapping table. The corresponding temperature change was then theoretically calculated using the equation (3.4). A few readings have been tabulated as shown in the temperature mapping table.

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTD</td>
<td>330 Ω</td>
</tr>
<tr>
<td>Heater</td>
<td>760 Ω</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$V_{out}$</th>
<th>$\Delta$RTD</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 V</td>
<td>0.4 Ω</td>
<td>23°C</td>
</tr>
<tr>
<td>1 V</td>
<td>19.8 Ω</td>
<td>41.4°C</td>
</tr>
<tr>
<td>2 V</td>
<td>39 Ω</td>
<td>60.5°C</td>
</tr>
</tbody>
</table>
Control and melting curves

Oil encapsulation
The control curve was obtained by heating a 5 μL droplet of mineral oil. The average of three experimental trials was plotted as seen in Figure 5.11a. The heater was ramped from 1 – 7 V in incremental steps of 0.1 V with a delay value of 10 s between each incremental step. The total run time was less than 11 min.

Figure 5.11: Control and melting curves. (a) The linear response of the mineral oil control curve showed no enthalpy changes during the temperature scan (b) The energy $Q$ used to melt an 800 μg sample of wax was demonstrated to be 0.176 J. The detected temperature change was $\approx 7.4 \, ^\circ\text{C}$. 

Glycerin encapsulation
The control curve was obtained by heating a 5 μL droplet of glycerin as seen in Figure 5.12a. The heater was ramped from 1 – 7 V in incremental steps of 0.1 V with a delay value of 5 s between each incremental step. The total run time was less than 6 min.
Figure 5.12: Control and melting curves. (a) The linear response of the glycerin control curve showed no enthalpy changes during the temperature scan. (b) The energy $Q$ used to melt an 800 $\mu$g sample of wax was demonstrated to be 0.176 J. The detected temperature change was $\approx 8.3$ m$^\circ$C.

### 5.4.3 Demonstration of salmon DNA melting

A fresh device was used for salmon DNA melting experiments. A 5 $\mu$L droplet of salmon DNA suspended in glycerin was applied to the device during the melting experiments. An average $T_m$ value of 70$^\circ$C was obtained from the melting experiments.

#### Device parameters and temperature mapping

Temperature mapping was performed using methods described in section 5.4.2. Table 5.3 describes the device parameters while table 5.4 describes the RTD temperature mapping as shown below. The device described in the table was heated from room temperature to a maximum of 105$^\circ$C. Every 1 V change in $V_{out}$ indicated a temperature change of $\approx 17^\circ$C as shown in the temperature mapping table.

<table>
<thead>
<tr>
<th>Table 5.3: Device parameters</th>
<th>Table 5.4: Temperature mapping</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electrodes</strong></td>
<td><strong>Resistance</strong></td>
</tr>
<tr>
<td>RTD</td>
<td>338 $\Omega$</td>
</tr>
<tr>
<td>Heater</td>
<td>723 $\Omega$</td>
</tr>
<tr>
<td>0 V</td>
<td>2.1 $\Omega$</td>
</tr>
<tr>
<td>1 V</td>
<td>19.1 $\Omega$</td>
</tr>
<tr>
<td>5 V</td>
<td>33 $\Omega$</td>
</tr>
</tbody>
</table>
Control and melting curves

The control curve was obtained by heating a 5 µL droplet of glycerin. The heater was ramped from 1 – 7 V in incremental steps of 0.25 V with a delay value of 15 s between each incremental step. The heating was continued at a slower heating rate with the heater ramped from 7.1 – 10 V in incremental steps of 0.1 V with a delay value of 15 s between each incremental step. The average of three experimental trials was plotted as seen in Figure 5.13a. 5 µL droplet of salmon DNA suspended in glycerin was heated under similar temperature scan conditions. The average of two experimental trials is as shown in Figure 5.13b. A linear fit was applied to both the curves near the area of interest to highlight the melting curve as seen in the corresponding right panel images in Figure 5.13. The total run time was less than 21 min.

![Graphs showing control and melting curves](image)

(a) Control curve: 5 µL glycerin

(b) Salmon DNA melting: 200 µM

Figure 5.13: Control and melting curves. (a) The linear response of the glycerin control curve showed no enthalpy changes during the temperature scan and its corresponding linear fit (b) The energy $Q$ used to melt the salmon DNA sample was calculated to be 46.86 mJ. The corresponding temperature change detected was $\approx 5.6$ m°C.

84
5.5 Discussion and Conclusions

5.5.1 Wax: Associated temperature changes

The temperature changes measured were associated with the detection capability of each device type. In other words the detection capability of the glass-wheatstone bridge illustrated in section 4.5.2 demonstrated a temperature detection of 186 m°C for an 800 µg sample of wax while the film device detected 8.3 m°C change for the same volume of wax. The difference between the two sample models was not only the type of substrate material but also the sample application method. While the wax sample on the glass substrate was melted with respect to air the wax sample on the PI device was encapsulated by 10× its volume with glycerol or oil, both of which are significantly denser than air with twice as much specific heat capacity values. The encapsulations added thermal mass to the device which reduced the resolution of the temperature detection. Conducting the wax experiments with and without air as reference was important due to two specific reasons. Firstly the inclusion of encapsulations allowed us to detect the melting of salmon DNA which was not possible with the earlier device generations due to their higher thermal mass. Secondly the encapsulations allowed us to experimentally determine the damping factor associated with melting wax in the presence of a dense encapsulation medium.

5.5.2 Conclusion

The thermal mass of the device as a whole was reduced by replacing the glass substrate with a PI film and PDMS microfluidics with droplet encapsulations. The device was successfully tested with 0.5 µL of 200 µM salmon DNA. Experiments with a lower DNA concentration yielded response curves similar to the control curves thus demonstrating a detection limit of ≈ 5.6 m°C. The temperature detection damping factor introduced due to the glycerol/oil encapsulation was ≈ 23× when compared to the in-air heating of wax on the glass-wheatstone device.

5.5.3 Limitations of current design

The device was unable to detect the melting of 0.5 µL of 200 µM salmon DNA suspended in 1×TE buffer encapsulated by oil or glycerin. The drawbacks associated with a single well detection system was that the test samples were compared to their reference samples in mutually exclusive experiments. In other words the single well experiments detected output changes with respect to ground. The use of differential measurement would enable measuring two floating inputs to the electronic capture system thus yielding an increased detection capability.
Chapter 6

Generation IV: Double Droplet Differential Detection with Thermal Equilibrators

Two heater-RTD pairs were used to derive differential measurements from generation IV devices. The double-well provided the means to measure the differential response between the reference and sample droplets encapsulated within oil. Copper islands called ‘thermal equilibrators’ were patterned directly under the heater-RTD pairs on the nickel-free side of the PI film to enable faster equilibration between all the active device components involved in contributing to the overall thermal signature viz. PI substrate, heater-RTD pair and the sample-oil droplets. The heater was supplied with a customized computer program to deliver heat using a constant power ramp. These new power settings on the heater helped in avoiding quadratic dependence of the RTD output voltage with respect to the heater input.

6.1 Background

6.1.1 Thermal equilibrators

Thermal equilibrators enhance the speed and uniformity of temperature spread over a designated area. Many research teams in the past incorporated a variety of thermal equilibrators to perform experiments rapidly and minimize baseline drift [187]. Isothermal titration calorimetry (ITC) employs thermal equilibrators to enhance the speed of temperature equilibration. ITC involves calorimetrically titrating one reagent with another to measure the thermodynamics of macromolecules in solutions at isothermal conditions [222]. The presence of thermal equilibrators hasten the homogenization process and enable speedy measurement. Torres et al. [271, 233]
developed enthalpy arrays by fabricating copper thermal equilibrators directly under the sample droplets while others maintained thermal equilibrium in flow calorimetry \cite{225} by injecting pre-heated samples into the calorimeter by using a negative feedback loop to control an external thermostat \cite{228} or a common water bath \cite{201}. Commercial ITC instruments also use thermal equilibrator blocks for thermostatization (Micro-ITC by Microcal, USA and Nano-ITC by TA Instruments, USA). Unlike the isothermal calorimeters which operate at thermal equilibrium conditions as described above heat capacity calorimeters operate at changing temperatures. The temperature changes that are induced due to resistive heating must be measured when the temperature is homogeneous over the entire sample volume. A straight-forward solution would be to include a mechanical stirrer within the sample. However this approach is not pursued in this project as mechanical stirring could add to joule heating that is hard to isolate for temperature measurements of the calorimetric system \cite{187}.

In this project copper equilibrators were used to compensate the low thermal conductivity of the PI substrate. The copper islands patterned on the bottom side of the PI substrate enabled each heater-RTD pair to minimize the time taken to reach equilibrium between each other and to homogenize the temperature profile within the sample droplet. The copper also helps to dissipate excess heat evenly to the environment. Such a type of heat dissipation mechanism was important for high-temperature ($\geq 90^\circ$C) biochemical reactions wherein the heater would be driven by high current densities to produce high temperatures. Without heat sinks and conductive heat dissipators the heater would tend to overheat dangerously causing irreversible damage. On the other hand the heat loss occurring due to the heat dissipation increases the power requirements of the microcalorimeter.

### 6.2 Materials and Methods

#### 6.2.1 Experimental setup

A block diagram of the experimental setup is as shown in Figure 6.1. The wheatstone bridge was connected to the signal conditioning unit and comprised two resistors from the device forming the left arm of the bridge. Figure 6.2(a) shows the positioning of the sensing RTDs within the wheatstone bridge. Fixed 2-wire resistors $R_3$ and $R_4$ completed the bridge along with a 100 $\Omega$ potentiometer for balancing the bridge to a null point. Figure 6.2(b) shows that the heaters from the two devices are shorted at one end before connecting to a DC power supply similar to a voltage divider circuit. Both sensing RTDs $R_1$ and $R_2$ of the device were incorporated into the wheatstone bridge on the breadboard. The electrical connections and interfacing between the different modular chip electronics and electrical instruments were similar to those described in section 5.3.1.
6.2.2 Buffers and reagents

A 12-mer dsDNA sample (Integrated DNA Technologies, Iowa, USA) with the sequence 5’ - CGCGAATTCGCG was used to acquire optical melting curves for demonstrating the capability
of the device for use as melting device with droplet samples. A stock solution of 742 µM solution was prepared by suspending the dsDNA in 1× TE buffer (IDT, USA). The stock solution was diluted into 10, 1 and 0.1 µM solutions by serial dilution. Four microliters of EvaGreen dye was used to tag 24 µL of the DNA suspensions. Steps described in section 3.3.2 were used to bind the dye with DNA.

6.2.3 Device fabrication

The microfabrication process employed was similar for steps 1 - 10 as described in section 5.3.3 for patterning nickel microelectrodes on the PI film. The following steps were involved to create an etching mask for copper for patterning equalizers on the nickel-free side of the PI substrate. Step 11 in Figure 6.3 shows that the PI film was re-mounted on the silicon wafer support with

---

**Figure 6.3:** Process flow for microelectrode fabrication on polyimide film with copper equalizers. (1) Clean copper-clad PI substrate (2) Spin coat 1.5 µm thick photoresist on surface (3) Soft bake at 95°C (4) Expose to UV light through a photomask to yield (5) Underexposed portions in the photoresist (6) Image reversal bake cross-links exposed photoresist pattern (7) Flood expose without a mask to expose remaining photoresist (8) The flood-exposed photoresist pattern was developed (9) Nickel is evaporated on the substrate surface (10) Remove photoresist using a solvent and turn over the substrate (11) Spin coat positive photoresist Shipley 1813 on the copper-side of the substrate and bake at 115°C for 1 min (12) Expose to UV light through a photomask to yield (13) Exposed portions in the photoresist (14) The photoresist was developed to leave behind the unexposed areas (15) Turn over and spin-coat Shipley 1813 to mask the nickel microelectrodes and introduce to a ferric chloride based copper etchant (16) Remove photoresist using acetone and rinse experiment-ready device with IPA.

89
the copper-side facing the user. Positive photoresist Shipley 1813 was spin-coated at 3000 rpm for 60 s on a Laurell spin coater for a final thickness of 2 µm. This is treated with a UV dosage of 70 mJ/cm². Since the exposed areas in a positive resist wash away upon developing the unexposed photoresist leaves a mask pattern on the copper protecting it from the etchant. The substrate is re-mounted again with the nickel side of the substrate for coating with a protective layer of Shipley 1813 and hard-baked at 115°C for 2 min before introducing to the etchant bath. The final device had square 3 × 3 mm copper equalizers positioned under the isolated heater - RTD pairs.

**Mounting the device**

The finished device was then mounted on a custom acrylic jig as seen in Figure 6.4. Mechanical rigidity was accomplished by stretching the film device over the acrylic jig and securing the ends with masking tape. This tensile stress caused by the stretching inhibited the RTD sensors from measuring resistance changes due to surface stress factors induced by external agents besides heater induced temperature changes. The two copper islands on the substrate were coated with teflon as described in section 5.3.3 for providing an oleophobic/hydrophobic surface for the droplet samples encapsulated by oil.

![Micrograph of the differential detection device](image)

**Figure 6.4:** Micrograph of the differential detection device: The heater - RTD pairs were separated by a lateral distance of 4 mm and were thermally isolated from each other due to individually assigned copper equalizer pads on the nickel-free side of the PI substrate.

**6.2.4 Heater input: Constant power generation**

Custom programming in Labview allowed precision control over the power input to the heater. Power was raised in step increments of the square-root of input voltage to minimize its quadratic
dependence on current. Figure 6.5 illustrates the use of Labview for designing the virtual instrument to control the DC power supply through a GPIB cable. The program allowed the user to select a start and an end voltage value depending on the final temperature desired. A ramp generator function ramped the voltage values in incremental steps. A timer delay function $\delta_t$ was included to set the dwell-time of each step increment.

![Figure 6.5: Schematic illustration of experimental setup for controlled heating: Labview generated constant heater power program featuring user-friendly control of variables. Delay time $\delta_t$ controlled the speed of the incremental steps.](image)

**Determination of time response**

The time response $\tau_{\text{air}}$ of a device was calculated as follows. The heaters of a blank device without any samples were given a step input of less than 2 V and were kept ON for 10 min while the DAQ recorded the output voltage value $V_{\text{out}}$ from the signal conditioning circuit at a sampling rate of 1 kHz. A step input of less than 2 V ensured that the tandem heaters would not burn out due to the excessive current density produced while heating air. Similarly TE droplets were placed as the reference samples on both heaters encapsulated by oil to calculate $\tau_{\text{ref}}$. $\tau_{\text{air}}$ and $\tau_{\text{ref}}$ were calculated from the voltage vs. time graph as the time taken for the output $V_{\text{out}}$ to reach 63% of its final value.

**Temperature mapping, control and sample curves**

For all experiments the heater was ramped from 0 – 12 V in steps of 0.22 s with a $\delta_t$ of 0.1 s. The response of the device with a 5 $\mu$L droplet of glycerin was recorded for 150 s as the control curve while a constant power ramp was applied to the heater.

In a separate experiment a DMM recorded the RTD resistance changes for the same volume of glycerin and the temperature was raised with scan settings similar to the control experiment.
to a final value of 73°C. The heater was not raised further in temperature as the supplier reported $T_m$ for wax was 55°C. The temperature map obtained from the DMM measurements provided the necessary target data points required for accurate mapping of the response.

Two samples of wax were molded and cut to weigh ≈ 160 µg and ≈ 50 µg. Each wax fleck was encapsulated by 5 µL of oil to emulate a setup with an evaporation-prone liquid sample. The RTD temperature map was used to determine the $T_m$ value for each sample.

6.2.5 Fluorescence validation experiments

Fluorescence experiments were performed following dsDNA tagging methods described in section 3.3.2. A glass coverslip device was used in lieu of the PI device as it was optically transparent. 1 µL of the EvaGreen tagged dsDNA was placed on the heater and encapsulated with 5 µL of oil. The heater was ramped from 0 – 6 V on the upscan and from 5.5 – 0 V on the downscan in steps of 0.5 V with a $\delta_t$ value of 30 s. One image was captured every 30 s with an exposure of 500 ms. The heater was turned ON at the end of the fourth image. The total run time was 15 min. A DMM was connected to the RTD which recorded corresponding resistance changes simultaneously throughout the scan. These resistance measurements were used in mapping temperatures on the fluorescent curves obtained. The image data obtained from the fluorescent curves were normalized and differentiated using methods described in section 3.3.3.

6.3 Results

6.3.1 Constant power source

![Figure 6.6: Constant power input: For a 2 kΩ heater (a) Shows a linear power input rate for heating air (b) Shows near-linear power input rate when heating a 5 µL droplet of oil.](image)

Figure 6.6: Constant power input: For a 2 kΩ heater (a) Shows a linear power input rate for heating air (b) Shows near-linear power input rate when heating a 5 µL droplet of oil.
Power measurements from the heater are as shown in Figure 6.6. A large thin film heater of 2000 Ω resistance value was chosen for this experiment to demonstrate a heightened dynamic range of its power capability. The power characteristics were obtained by heating the 2000 Ω heater with an incremental ramp signal from a DC power supply controlled by a Labview program as outlined before. A maximum of 7.5 mA was used to heat up air in 4 min as seen in Figure 6.6(a) while 15 mA was used to heat up 5 µL of oil Figure 6.6(b) in 15 min. The difference in total run times for the air and oil experiments was due to the varying end voltages that the heater was ramped to. The power responses were optimized to have a linear dependence on time and the DC power supply variables applied are as seen in Figure 6.6. This large 2 kΩ heater exhibited a maximum power of 100 mW. In contrast a heater one-tenth the size would exhibit ≈ 10 mW of power generation.

6.3.2 DNA melting: Fluorescence validation
Three concentrations of dsDNA (10, 1 and 0.1 µM) were used to demonstrate DNA melting within the droplets placed on the device. Each test involved heating a 1 µL EvaGreen-bound DNA droplet encapsulated by 5 µL oil drop while recording its fluorescence intensity. Figure 6.7 shows the recorded fluorescence intensity values and their corresponding normalized values.

6.3.3 Demonstration of wax melting

Time response
Three repeatable experimental trials on an empty device yielded a stable response for 10 min as seen in Figure 6.8. The corresponding figure to its right shows the time response $\tau_{\text{air}}$ of 2 s. The inset in Figure 6.8 shows the heater input for the temperature scan. The time response for a 5 µL glycerin droplet was calculated similarly and found to be 4 s (not shown). The noise floor of the data sets was ≈ 1.5 mV.

Glycerin control and temperature mapping
The control experiment involved heating two identical 5 µL droplet samples of glycerin on each heater coil. The heater was ramped from 0 – 12 V in incremental steps of 0.22 V with a delay value of 0.1 s. The total time for each experiment was 6 min. The $V_{\text{out}}$ from the signal conditioning circuit was recorded via the DAQ at 1 kHz frequency and at a gain of 1000. The control curve increases steadily with temperature and did not exhibit anomalies as seen in Figure 6.9a. This indicated that heating the glycerin sample progressed without causing enthalpy changes or phase transitions. The noise floor was ≈ 1.5 mV. The RTD resistance changes were plotted as a function of temperature as seen in Figure 6.9b.
Figure 6.7: Fluorescence validation of DNA melting. The heater was ramped in steps of 0.5 V from 0 – 6 V for 15 min during the heating cycle and from 5.5 – 0 V for the cooling cycle with a delay time of \( \tau = 30 \) s between each incremental step.

**Wax melting**

The heater was ramped with power settings similar to the control experiments. The melting curves for various masses of wax showed melting points at \( \approx 55.4 \pm 0.6 \)°C as seen in Figure 6.10.
Figure 6.8: Determination of time response: The device showed a stable response as seen on the left. The area of interest during the initial heater device took 2 s to reach 63% of its final value as seen in the figure on the right. The fresh device used in this experiment had RTD values: 371 Ω and 353 Ω and heater values: 375 Ω and 350 Ω.

Figure 6.9: Thermal response of device: (a) Glycerin control curve showing a linear increase with temperature (b) Temperature profile of the device as seen by the corresponding RTD temperature map (n=3).

Figure 6.10: Melting characteristic of paraffin wax. The wax samples were encapsulated by a 5 μL glycerin drop (n=3). The energy $Q$ used to melt the wax samples was calculated to be (a) $Q = 35.2 \text{ mJ}$ and (b) $Q = 11 \text{ mJ}$. 

95
6.3.4 Demonstration of salmon DNA melting

A fresh device was used for salmon DNA melting experiments. Salmon DNA suspended in 1×TE buffer solution was prepared using methods described in section 5.3.2.

Time response and RTD temperature mapping

The total run time for the time response experiment was 10 min. The time response of an empty device was determined to be 2.1 s as seen in Figure 6.11a using methods described in section 6.3.3. A 3 μL salmon DNA droplet of concentration 10 μM was encapsulated in a 7 μL drop of oil. The heater was ramped from 0 – 16 V in incremental steps of 0.22 V with a delay value of 2.1 s. The δt value was increased 10× to account for the 2× sample volume increase. The DMM recorded the RTD R2 resistance values as seen in Figure 6.11b while the heater was ramped linearly.

![Figure 6.11: Time response and RTD temperature mapping](image)

(a) Time response of device  
(b) RTD temperature map

The device showed a time response of 2.1 s while heating air. The fresh device used in this experiment had RTD values: 428 Ω and 388 Ω and heater values: 443 Ω and 400 Ω. The device was heated from room temperature to a maximum of 94°C.

Control and melting curves

A temperature scan similar to the RTD temperature mapping trial was applied to obtain the control curve. The V_{out} from the signal conditioning circuit was recorded via the DAQ. The control curve increases steadily with temperature and did not exhibit anomalies as seen in Figure 6.12a. This indicated that heating the TE sample progressed without causing enthalpy changes or phase transitions. On the other hand the salmon DNA exhibited melting at 89°C as seen in Figure 6.12b. The noise floor was ≈ 1.5 mV.
Figure 6.12: Control and melting curves: (a) The linear response of the TE control curve shows no enthalpy changes during the temperature scan. (b) The energy \( Q \) used to melt the 10 µM salmon DNA samples was calculated to be \( Q = 1.4 \text{ mJ (} n=3 \text{)} \).

6.4 Discussion and Conclusions

6.4.1 Wax melting

Two different wax samples of varying masses were melted. The bigger sample of wax was 3× higher mass than the smaller sample. While both samples had matching trends in their melting curves it was also evident from the curves that the difference in their peak transition \( V_{out} \) values also scaled proportionally. The difference in the \( V_{out} \) value from the point of deflection toward a non-linear trend to the peak value of the 50 µg wax sample was \( \approx 3 \times \) less than that of the 160 µg sample. This showed that the device response was proportional to the thermal signatures of the different sample masses used.

6.4.2 Experimental determination of \( Q \)

The power delivered to the heaters by the DC power supply during the control and wax melting experiments were plotted as seen in Figure 6.13. The wax curve indicates the amount of power delivered to the heaters during the wax melting experiment and highlights the area of interest. The corresponding power delivered to the heaters during the temperature scan of the control curve was also plotted. The area under each curve was integrated using the trapezoidal rule of integration and the difference of integrals indicated the amount of input power \( Q \) delivered to the heaters for melting 160 µg of wax. The theoretical \( Q \) required to melt 160 µg of wax was 35.2 mJ which is in agreement with the experimentally determined value.
Figure 6.13: Experimental calculation of $Q$: The theoretical $Q$ calculated to be 35.2 mJ was in agreement with the experimentally derived value of 190 mJ.

6.4.3 Conclusions

Generation IV devices were able to successfully detect the melting of 10 µM salmon DNA samples. The detection of lower concentration DNA samples (i.e. 1 µM) showed insignificant melting peaks and hence were not included in the report. With optimized constant power input, signal conditioning circuitry and double-well differential detection we improved resolution by $40 \times$. In order to improve the limit of detection the thermal mass of the samples could be reduced further. The following chapter explains the modifications applied in the droplet placement techniques for enhancing the detection capabilities.
Chapter 7

Generation V: 3D Double Droplet Differential Detection

The total heat capacity of the double droplet device system was minimized even further by reducing the combined oil-sample droplet volumes by 85%. The droplets were created by encapsulating a 0.4 µL sample droplet within an equal volume of oil. Sample evaporation was controlled by placing a 12.5 µm thick PI lid supported by spacers to form a 3D device structure. With its low thermal mass the PI lid not only contributed insignificantly to the total heat capacitance of the overall system but its surface tension properties also helped mechanically immobilize the droplets in a 'spread-out' fashion on the heater for uniform heating. Four model systems - PFD beads, lysozyme, salmon DNA and mammalian DNA were melted successfully demonstrating a detection capability.

7.1 Background

7.1.1 Lysozyme

Lysozyme is a globular protein with a low molecular weight of 14307 Da [35, 47]. Since its discovery by Fleming in 1922 it has been one of the most investigated proteins [130, 261] as it can be easily isolated, purified and stabilized for long-term storage [35]. Lysozyme is abundantly available in hen egg whites which serves as an antibiotic as well as a nutritional resource for a developing chick embryo [247].

The enthalpy of transition $\Delta H$ of lysozyme suspended in a variety of buffers has been reported by research groups in the past. While $\Delta H$ can be as low as 86 kJ/mol when suspended in dodecyltrimethylammonium bromides (DTABs) or phosphate buffer saline (PBS) [17], it can be 125 kJ/mol in guanidine hydrochloric acid [10] and higher at 160.81 kJ/mol in sodium
dodecyl sulphate (SDS) solutions [18]. Similarly the melting temperatures also vary with varying solvents [142]. In this project the enthalpy of unfolding of lysozyme due to thermal melting was estimated as $\approx 500 \text{ kJ/mol} [56]$.

Renaturation of lysozyme has been reported to be incomplete [126, 80] upon cooling. Consequently when the same lysozyme sample was reused the resulting $\Delta H$ of the second temperature up-scan yielded 48% less than the initial $\Delta H$ value [80] and lower fluorescence intensity [126]. Reconstituting lysozyme with additives such as glycerol, glucose, ammonium sulphate etc post the initial renaturation was reported to improve the quality of the folding [177]. The inability of lysozyme to completely undergo renaturation may be attributed to the exposure to temperature greater than or equal to 100$^\circ$C [126] causing irreversible damage to the component amino acids. In this project fresh samples of lysozyme were used in every trial and were not reused for maintaining uniformity in heat capacity calculations throughout all experimental sets.

### 7.1.2 PFP bubbles

Dodecafluoro-n-pentane ($\text{C}_5\text{F}_{12}$) or better known as perfluoropentane (PFP) is a fluorocarbon with a density of 1.644 g/mL and molecular weight of 288 g/mol [132]. It is a nontoxic fluoroalkane that is highly hydrophobic. Due to its high hydrophobicity PFP exhibits a high interfacial tension when dispersed in hydrophilic media such as water. Additives such as surfactants have been reported to improve its dispersion in aqueous media and stabilize the immiscible emulsion of PFP bubbles in solution. Such biocompatible emulsions have found varied applications in medical therapies and breakthrough biomedical applications [236, 234, 274, 229]. Due to its high affinity to oxygen PFP bubbles have been employed for target blood vessel occlusion near tumor sites [149] and as synthetic carriers of oxygen [173, 172, 109, 105, 230]. PFP bubbles have been used as propellants in pressurized inhalers for direct drug delivery to the lungs [249].

As the boiling point of PFP bubbles lies between room temperature and body temperature their use as drug delivery vehicles [84, 254, 167] has extensively expanded into invivo applications. With control over stabilizing emulsions via surfactants and lipids PFP bubbles have been used as ultrasound contrast agents and in investigative radiology [141, 275, 169].

For this project liquid PFP was dispensed into water to create an emulsion. The surface tension of the water provided sufficient interfacial wall tension for the formation of PFP bubbles within the water. Most bubbles assumed a spherical shape in order to minimize the surface area of interaction with the water. The PFP bubbles formed had average radii varying between 200 – 1µm. Surface tension and the bubble radius play an important role in stabilizing pressure differences between the inside and outside of the bubbles. While the bubble’s internal pressure pushed the walls outward a counteractive surface tension force acted on its outer surface area keeping the bubble from exploding. This follows the Laplace law where counteractive forces
enable the existence of gas or liquid bubbles within a liquid medium.

7.2 Materials and Methods

7.2.1 Device fabrication

Methods described in section 5.3.3 were used to fabricate the PI film device. The inner coil was used as the heater while the outer coil was used as the RTD as illustrated in Figure 7.1

![Figure 7.1: Micrograph of differential detection device illustrating the new RTD and heater allocations.](image)

7.2.2 3D-device assembly

The 3D device composed three major components viz. teflon-coated PI films, PDMS spacers and the heater-RTD pair devices fitted with copper-equilibrators. A schematic of the cross-section of the entire device is as shown in Figure 7.2. The copper-side of the final PI film device and a plain 12.5 µm PI film were coated with 50 nm of teflon. The PI substrate with the nickel heater-RTD pairs and copper islands was first fastened using masking tape on a custom acrylic jig. Samples of commercially available 250 µm thin PDMS membranes were obtained (SSP-M823, SSP, USA). A 2 × 2 mm square window was cored out using a fine-tipped X-acto knife (Elmer’s Products Inc. Ohio, USA). The PDMS window was chiseled further to make ≈ 250 µm wide frame. This ensured that the window did not cover the coils of the heater-RTD pair or the copper and was light-weight so not to cause mechanical stresses on the surface of the PI film.
Two such identical PDMS windows were placed on the device one for each heater-RTD pair to form the spacers. After the deposition of the droplets the teflon-coated PI films were placed on the droplets carefully with the help of a pair of fine-tipped tweezers. The PDMS spacers along with the two teflon-coated PI films formed the 3D device.

![Diagram of 3D double droplet system](image)

Figure 7.2: Schematic: Cross-section of 3D double droplet system. *(Not to scale)*

### 7.2.3 Droplet positioning

A 0.4 μL droplet of the liquid sample was first placed on the teflon-coated copper-side of the PI device. A 0.4 μL of oil was then carefully positioned to encompass the sample droplet uniformly from all directions. Both droplets were initially deposited on the device using micro-pipettes. Further on-chip manipulation of the droplets was done manually using a human eyelash. A latent image of the outer ring of the RTD was visible from the copper side of the substrate in every device and aided droplet manipulation for precision positioning. The top of the liquid sample remained exposed as the volume of oil used was insufficient to encapsulate the sample droplet fully. The teflon-coated PI film was then placed as a lid on the droplet supported by the PDMS spacers using a pair of fine-tipped tweezers. The liquid and oil droplets spread out under the weight of the PI film on top and their combined outer diameter spread to the space in between the heater and RTD. Care was taken not to allow the droplets to spread to the RTD. This ensured that the thermal transmission from sample to the RTD occurred laterally through the substrate and not through the overlapping droplet. Thus both the teflon-coated PI films faced the liquid samples positioned between them to create a hydrophobic drop which
spread out evenly over the heater area as seen in Figure 7.3.

![Figure 7.3: Photograph of 3D-droplet device illustrating a 0.4 μL droplets of the sample encapsulated by a 0.4 μL droplets of oil. The PDMS spacers were positioned on the substrate such that they do not make contact with the copper equilibrators.](image)

### 7.2.4 Experimental protocol for different melting models

The 3D double-droplet differential detection scheme was used to obtain melting characteristics of various organic model materials capable of exhibiting enthalpy change during a phase transition. These melting experiments were preceded by important control experiments in order to analyze accurate thermal parameters of the device under various input conditions. Such preceding experiments were conducted following an ordered step process to calculate the parameters as enlisted below.

1. Time response of an empty chip ($\tau_{\text{air}}$)
2. Time response of the reference sample ($\tau_{\text{ref}}$)

3. RTD temperature mapping

4. Control buffer curves

5. Sample melting curves

The time responses were calculated to understand the optimal speed for operating the device. Resistance values from the RTD were recorded with a digital multimeter (DMM) with a reference sample to map the corresponding temperature of the scan. Consequently melting experiments were performed on the model materials and data were collected via the DAQ at a sampling rate of 1 kHz. The heater was ramped with constant power as illustrated in section 6.5. The control and melting experiments were performed as sets of three trials each to ensure repeatability between individual runs and the results reported in this thesis display an average of the three data sets unless stated otherwise.

Every model material was tested on a fresh device after determining the thermal parameters of that device. Although all the devices were fabricated in a batch process the heater and RTD resistance values of devices in the same batch varied on an average of 10%.

A fresh substrate was used for each type of sample.

7.2.5 Buffers and reagents

Lysozyme (L7651, Sigma Aldrich, USA) of molecular mass 14307 g/mol was used to make a stock solution of 100 µM by suspending 143 mg of the enzyme in 100 µL of DI water. 10 µM and 1 µM concentrations were prepared by serial dilution of the stock solution. The enzyme was thoroughly vortexed to enable uniform mixing. The samples were then subjected to centrifugation for $\approx 1 - 2$ min to remove trapped air bubbles and refrigerated at 4°C. The samples were vortexed momentarily before applying the droplets to the device for melting.

PFP bubble suspension were created by dispersing liquid PFP (1100-2-05, SynQuest Laboratories, FL, USA) in water. $\approx 1$ mL of the liquid PFP was added to $\approx 5$ mL of water and shaken vigorously to break up the gas into micron-sized spherical bubbles. The radii of the bubbles ranged from 200 – 1 µm. The sample container was refrigerated 4°C to avoid spontaneous explosion of the volatile gas bubbles whose supplier reported boiling point ranges from 26 – 35°C.

Purified 12-mer dsDNA sample (Integrated DNA Technologies, Iowa, USA) with the sequence 5' - CGCGAATTCGCG with molar mass 804 nM was used to acquire melting curves. A stock solution of 75 mM was prepared by adding 10.75 µL nuclease-free 1× TE buffer (30 mM Hepes, pH 7.5, 100 mM potassium acetate, IDT, USA) directly to the centrifuge tube.
shipment. Consequently 7.5 mM and 0.75 mM solutions were prepared by serial dilution from the stock solution.

7.3 Results

7.3.1 Demonstration of salmon DNA melting

A fresh device was used for salmon DNA melting experiments. Three concentrations of salmon DNA (10, 1 and 0.1 µM) were prepared using methods described in section 5.3.2. Applying the universal experimental protocol described in section 7.2.4 the following thermal characteristics of the device were determined.

Time response curves (τ) and RTD temperature mapping

![Time response curves](image)

Figure 7.4: Time response curves: (a) The device showed a stable response while heating air. The corresponding area of interest shows that the device takes 0.6 s as its τair to reach 63% of its final value (b) The device was loaded with 0.4 µL droplet of TE encapsulated by 0.4 µL of oil. The corresponding area of interest shows that the device took 3 s to reach 63% of its maximum value (c) The fresh device used in this experiment had RTD values: 352 Ω and 270 Ω and heater values: 356 Ω and 330 Ω. The device described was heated from room temperature to a maximum of 91°C.
The heater was given a step input of 1.73 V for 10 min while the $V_{\text{out}}$ was recorded from the DAQ. The time response of an empty device $\tau_{\text{air}}$ was determined to be 0.15 s as seen in Figure 7.4a. that of a typical sample i.e. 0.4 µL droplet of TE encapsulated by 0.4 µL of oil was 3 s as seen in Figure 7.4. The $\tau_{\text{air}}$ of the device was determined with the PDMS spacers and the teflon-coated PI film. The noise floor was $\approx 1 \text{ mV}$.

**Control and melting curves**

The control and experimental trials as seen in Figure 7.5 were performed under scan conditions similar to the RTD temperature mapping experiment described above. The $V_{\text{out}}$ from the signal conditioning circuit was recorded via the DAQ. While the control curve increased linearly without displaying phase transitions the melting curves for various concentrations showed melting points at $\approx 71.25 \pm 3{}^\circ\text{C}$.

![Control and melting curves](attachment:image.png)

Figure 7.5: Control and melting curves: a) The linear response of the TE control curve shows no enthalpy changes during the temperature scan (b) The 10 µM melting curve demonstrated the detection of $Q = 187.4 \mu\text{J}$ (c) The 1 µM melting curve demonstrated the detection of $Q = 18.74 \mu\text{J}$ (d) The 0.1 µM melting curve demonstrated the detection of $Q = 1.87 \mu\text{J}$. 

7.3.2 Demonstration of lysozyme denaturation

A fresh device was used for testing lysozyme melting. The device was characterized for thermal parameters and melting curves of three concentrations of lysozyme (100, 10 and 1 mM) are shown.

Time response curves ($\tau$) and RTD temperature mapping

The heater was given a step input of 1.73 V for 10 min while the $V_{\text{out}}$ was recorded from the DAQ. The time response of an empty device $\tau_{\text{air}}$ was determined to be 2 s while that of a typical

![Time response curves](image)

Figure 7.6: Time response curves: The time taken by the device to reach 63% of its maximum value was determined as $\tau$. (a) The $\tau_{\text{air}}$ of the device heating air was determined with an empty 3D PI enclosure. The corresponding area of interest shows the $\tau_{\text{air}}$ to be 0.2 s (b) The $\tau_{\text{ref}}$ represented the time response of a reference 0.4 µL droplet of DI water encapsulated by 0.4 µL of oil. (c) The fresh device used in this experiment had RTD values: 356 $\Omega$ and 342 $\Omega$ and heater values: 354 $\Omega$ and 240 $\Omega$. The device was heated from room temperature to a maximum of 78$^\circ$C.

...sample i.e. 0.4 µL droplet of TE encapsulated by 0.4 µL of oil was 4 s as seen in Figure 7.6. The $\tau_{\text{air}}$ of the device was determined with the PDMS spacers and the teflon-coated PI film. The noise floor was $\approx 1.5$ mV.

The temperature profile of the device as recorded by the RTD during a temperature scan on a typical sample is shown in Figure 7.6c. The heater was ramped from 0 – 10 V in incremental
steps of 1 V with a $\tau$ value of 4 s matching the $\tau_{ref}$ value obtained from the time response experiment described in the previous section. The total run time was 7 min. The table represents the electrode parameters of the device used.

Control and melting curves

![Control curve](image1.png)
![Melting curve of 100 mM lysozyme](image2.png)
![Melting curve of 10 mM lysozyme](image3.png)
![Melting curve of 1 mM lysozyme](image4.png)

Figure 7.7: Control and melting curves: a) The linear response of the DI control curve shows no enthalpy changes during the temperature scan. (b) The 100 mM lysozyme melting curve demonstrated the detection of $Q = 20 \text{ mJ}$. (c) The 10 mM melting curve was fitted with a straight line to enhance the melting response. The melting demonstrated the detection of $Q = 2 \text{ mJ}$. (d) The curve shows a representative response of 1 mM lysozyme. Linear fit was applied to each of the three repeatable trials for calculating the mean $T_m$. The response demonstrated the detection of $Q = 0.2 \text{ mJ}$.

The control and experimental trials as seen in Figure 7.7 were performed under scan conditions similar to the RTD temperature mapping experiment described above. The $V_{out}$ from the signal conditioning circuit was recorded via the DAQ. While the control curve increased linearly without displaying phase transitions the melting curves for various concentrations showed melting points at $\approx 59.15 \pm 3.5 \degree C$. The 1 mM curve was baseline subtracted to aid visual representation of the melting. The average of three trials was subtracted from the average of the control curve to produce Figure 7.7d.
7.3.3 Demonstration of 12-mer DNA melting

A fresh device was used for DNA melting experiments. The device was characterized for thermal parameters and melting curves for three concentrations of the 12-mer DNA (75, 7.5 and 0.75 mM are shown.

Time response curves ($\tau$)

The heater was given a step input of 1.73 V for 10 min while the $V_{out}$ was recorded from the DAQ. The time response of an empty device $\tau_{air}$ was determined to be 0.12 s as seen in Figure 7.8a. The $\tau_{ref}$ typical sample i.e. 0.4 µL droplet of TE encapsulated by 0.4 µL of oil was 0.3 s as seen in Figure 7.8b. The $\tau_{air}$ of the device was determined with the PDMS spacers and the teflon-coated PI film. The noise floor was $\approx$ 1 mV.

Figure 7.8: Time response and device parameters: (a) The time taken by an empty device $\tau_{air}$ to reach 63% of its maximum voltage value was determined to be 0.12 s (b) The time taken by a loaded device $\tau_{ref}$ to reach 63% of its maximum voltage value was determined to be 0.3 s. The fresh device used in this experiment had RTD values: 298 Ω and 284 Ω and heater values: 285 Ω and 277 Ω.

RTD temperature mapping and control curve

The temperature profile of the device as recorded by the RTD during a temperature scan on a typical sample is shown in Figure 7.9 (a). The heater was ramped from 0 – 8 V in incremental steps of 0.25 V with a $\tau$ value of 0.3 s matching the $\tau_{ref}$ value. The total run time was $\approx$ 90 s. The control curve is as seen in Figure 7.9 (b) were performed under scan conditions similar to the RTD temperature mapping experiment described. The $V_{out}$ from the signal conditioning circuit was recorded via the DAQ.
Figure 7.9: RTD temperature mapping and control curve: (a) The device was heated from room temperature to a maximum of 56°C (b) The linear response of the TE control curve shows no enthalpy changes during the temperature scan.

12-mer DNA melting curves

The melting curves for all concentrations showed melting points at $\approx 48.1^\circ$C as seen in Figures 7.10a, 7.10b and 7.10d. The melting curve of the lowest concentration 0.75 mM as seen in Figure 7.10 (d) was coupled with a linear fit at the area of interest to enhance the visual representation of the melting event. In addition the TE control curve also coupled with a linear fit has been showcased in the same figure panel (Figure 7.10 (c)) under identical image post-processing conditions.
7.3.4 Demonstration of PFP bubble boiling

A fresh copper-free double differential PI device was used to demonstrate PFP bubble boiling. The device was characterized for thermal parameters and boiling curves PFP bubbles are shown.

Time response curves (τ) and RTD mapping

The heater was given a step input of 1.73 V for 10 min while the $V_{out}$ was recorded from the DAQ. The time response of an empty device $\tau_{air}$ was determined to be 0.1 s as seen in Figure 7.11a. The $\tau_{ref}$ of a typical sample i.e. 0.4 µL droplet of TE encapsulated by 0.4 µL of oil was 1.5 s as seen in Figure 7.11b (b). The $\tau_{air}$ of the device was determined with the PDMS spacers and the teflon-coated PI film. The noise floor was $\approx$ 1 mV. The temperature profile of the device as recorded by the RTD during a temperature scan on a typical sample is shown in Figure 7.11c. The heater was ramped from 0 – 3.5 V in incremental steps of 0.25 V with a $\tau$ value of 1.5 s matching the $\tau_{ref}$ value obtained from the time response experiment described in
Figure 7.11: Time response curves: The time taken by the device to reach 63% of its maximum value was determined as $\tau$. (a) The $\tau_{\text{air}}$ of the device heating air was determined with an empty 3D PI enclosure. The corresponding area of interest on the right shows $\tau_{\text{air}}$ value of 0.1 s. (b) The $\tau_{\text{ref}}$ represented the time response of a reference 0.4 $\mu$L droplet of TE encapsulated by 0.4 $\mu$L of oil. $\tau_{\text{ref}}$ was determined to be 1.5 $\mu$L. (c) The fresh device used in this experiment had RTD values: 219 $\Omega$ and 211 $\Omega$ and heater values: 212 $\Omega$ and 216 $\Omega$. The device was heated from room temperature to a maximum of 34°C. (d) The control and boiling curves as seen in Figure 7.12 were performed under scan conditions similar to the RTD temperature mapping experiment described above. The $V_{\text{out}}$ from the signal conditioning circuit was recorded via the DAQ. While the control curve increased linearly without displaying phase transitions. The boiling curves showed boiling at temperatures ranging from 27 – 32°C. Phase transition events depicted in the boiling curves were representative of the boiling of gas bubbles. The average of three control trials has been displayed as the representative control curve as seen in Figure 7.12 (a). However the boiling curves of three individual PFP trials have been presented in Figures 7.12b, 7.12c and 7.12d. The average of the PFP curves was not considered as the bubble radii and concentration in each sample were random and could not be controlled. Hence the wide range of boiling temperatures.

the previous section. The total run time was 90 s.
Figure 7.12: Control and boiling curves: (a) The linear response of the DI control curve shows no phase changes during the temperature scan. (b), (c), (d) The curves on the left panel demonstrate the boiling events while the curves on their right represent their corresponding boiling peaks also represented by $T_m$. 

(a) Control curve

(b) Example 1: Boiling of PFP bubbles

(c) Example 2: Boiling of PFP bubbles

(d) Example 3: Boiling of PFP bubbles
7.4 Discussion

7.4.1 Thermal capacitance of device

The thermal capacitance of the calorimeter was calculated using the constants described in the table 7.1 below. The combined heat loss \( Q_{\text{total}} \) from the device was calculated from equations (1.7a) and (1.7b).

\[
Q_{\text{total}} = Q_{\text{air}} + Q_{\text{subs}} = 142 \text{ } \mu\text{W/K} \tag{7.1}
\]

The time response \( \tau_{\text{ref}} \) of the PFP device as seen in section 7.3.4 was experimentally determined to be 1.5 s. Since the resistance \( R \) of the system can be represented as the inverse of its conductance or the ability of dissipate heat, the thermal capacitance \( C_{\text{device}} \) of the device as defined in equation (7.2) was calculated to be

\[
C_{\text{device}} = \tau_{\text{ref}}/R = 47.12 \text{ } \mu\text{J/K} \tag{7.2}
\]

7.4.2 Copper vs. copper-free differential devices

Copper-free devices were used for demonstrating PFP bubble boiling and copper equilibrator devices were used for demonstrating melting of salmon DNA, lysozyme and 12-mer DNA.

<table>
<thead>
<tr>
<th>Component</th>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate (PI)</td>
<td>Thickness</td>
<td>12.5 ( \mu\text{m} )</td>
</tr>
<tr>
<td>Substrate (PI)</td>
<td>Length</td>
<td>10 mm</td>
</tr>
<tr>
<td>Heater (Ni)</td>
<td>Length</td>
<td>1 mm</td>
</tr>
<tr>
<td>Equilibrator (Copper)</td>
<td>Length</td>
<td>3 mm</td>
</tr>
<tr>
<td>PI</td>
<td>Conductivity ( \kappa_{\text{PI}} )</td>
<td>0.52 Wm(^{-1})K(^{-1})</td>
</tr>
<tr>
<td>Air</td>
<td>Conductivity ( \kappa_{\text{PI}} )</td>
<td>0.0257 Wm(^{-1})K(^{-1})</td>
</tr>
<tr>
<td>Copper</td>
<td>Conductivity ( \kappa_c )</td>
<td>401 Wm(^{-1})K(^{-1})</td>
</tr>
</tbody>
</table>
Using parameters mentioned in Table 7.1 heat loss due to conductance through the copper equilibrators $Q_c$ was included for calculating the total heat loss $Q_{\text{total}}$ as seen in equation (7.3)

$$Q_{\text{total}} = Q_{\text{air}} + Q_{\text{subs}} + Q_c = 20 \text{ mW/K} \quad (7.3)$$

Consequently for the copper-based devices the thermal capacitance was calculated using the equation (7.4) with $\tau_{\text{ref}} = 0.3 \text{ s}$ as obtained for the 12-mer DNA device described in section 7.3.3

$$C_{\text{device}} = \frac{\tau_{\text{ref}}}{R} = 6 \text{ mJ/K} \quad (7.4)$$

The copper-free devices possessing a total thermal capacitance value of 47.12 $\mu$J/K were more favorable than their copper-based counterparts due to their higher detection capability. A photograph of the experiment-ready devices is shown in Figure 7.13.

![Figure 7.13: Photograph: Copper and copper-less devices. The thermal capacitance of the copper-less devices was an order less than that of the copper devices.](image)

However the copper-free devices failed at temperatures ranging from $\approx 40–95^\circ \text{C}$ for melting salmon DNA, 12-mer DNA or lysozyme. The nickel traces patterned on the low conductivity PI
membrane served as a poor dissipator of the heat generated by the heater causing high current densities to flow through the heater traces. The undesirable effects of high current densities include driving the heater beyond a finite maximum resistance and causing electromigration - both leading to irreversible damage to the heater rendering it open-circuited.
Chapter 8

Conclusions and Future Work

A portable melting device with rapid response time and minimal sample volume consumption was designed and tested successfully with four different types of melting models. Carefully devised fabrication techniques coupled with custom-designed electronic circuitry resulted in creating a microcalorimetric biosensor capable of detecting enthalpy changes in the order of 2 µJ. The melting temperatures detected varied from the supplier reported $T_m$ values by $\approx \pm 3^\circ C$. A correction factor based on the heat capacity value of each type of microcalorimeter was supplied for higher accuracy.

An overview of the work done with relevant conclusions are presented in this chapter. Theories on future directions for the project are put forth.

8.1 Summary

The energy consumed $Q$ by the sample to result in enthalpy change and its corresponding temperature change $\Delta T$ as detected by the each device has been summarized in Table 8.1. The

<table>
<thead>
<tr>
<th>Generation</th>
<th>Device type</th>
<th>$\tau$</th>
<th>$Q$</th>
<th>Noise floor</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Glass-PDMS</td>
<td>19 s</td>
<td>Optical MCA</td>
<td>20 mV</td>
</tr>
<tr>
<td>II</td>
<td>Glass-Wheatstone bridge</td>
<td>18 s</td>
<td>0.176 mJ</td>
<td>20 mV</td>
</tr>
<tr>
<td>III</td>
<td>Single-well PI film</td>
<td>2 s</td>
<td>46.86 mJ</td>
<td>2 mV</td>
</tr>
<tr>
<td>IV</td>
<td>Double-well PI film</td>
<td>2 s</td>
<td>187 µJ</td>
<td>1.5 mV</td>
</tr>
<tr>
<td>V</td>
<td>3D double-well with copper</td>
<td>2 s</td>
<td>1.87 µJ</td>
<td>1 mV</td>
</tr>
</tbody>
</table>

Table 8.1: Performance Metrics
table shows the progressive improvement in the resolution of temperature detection due to the reduction in thermal mass.

### 8.1.1 Research contributions

One of the novel contributions of this research is the technique of pressurizing the reaction chamber without affecting the experimental sample and as a means to increasing signal to noise ratio at the data acquisition end due to sample containment without interference of bubbles or rapid evaporation. Such a pressurization technique is applicable to any melting/PCR device composing a rigid substrate such as glass, silicon etc. integrated with PDMS or other polymer based microfluidics. The project also highlights the process of minimizing sample volumes to approximately half of that applied in traditional droplet microfluidics by incorporating the 3D technique of oil encapsulation. The device is operable while freely suspended in air and does not require vacuum for optimal function. The microfabrication techniques presented in the thesis allow high volume production and further scaling of the device to achieve higher detection capabilities. In addition microfabrication would enable the creation of a high throughput design when the device is patterned as an array. The microcalorimetric device developed along with the modular electronics enables the electronic detection of DNA based on calorimetric measurements thus eliminating the need for expensive and bulky optical detection equipment.

### 8.1.2 Device engineering

Finite element modeling was used to determine the optimal electrode design which was employed universally in all device generations. The high performance microcalorimetric device presented in Chapter 7 is a culmination of five generations of predecessors. Each evolutionary stage was guided by three main principles:

1. To construct the device with cost-effective raw materials.
2. To devise fabrication methodologies entailing rapid prototyping or batch processing to enable mass production of disposable and cheap microcalorimeters. This would be advantageous for future automation.
3. To decrease the thermal capacitance of the device and hence increase detection sensitivity.

The device fabrication involved using off-the-shelf raw materials such as glass and PI films as substrate materials while cost-effective metals such as nickel and copper were used as microelectrodes and thermal equilibrators respectively. While the bulk construction materials were being constantly reduced in their thickness and percentage utilization in designing the device a similar scaling strategy was applied to reduce the sample volume.
8.1.3 Modular microelectronic circuit design

The detection of melting was based on electrical sensing which evolved from using commercially available electronic chip components to custom-design modular circuitry. Direct DMM readout from the device was transformed into incorporating the sensing RTD within a more sensitive wheatstone bridge to enhance the signal to noise ratio. Simultaneously the excitation mode was switched from DC to AC in order to process cleaner signals. The opamp based capture electronics complemented the single well design by providing means to acquire data at a high sampling rate. More sophisticated ICs such as the lock-in amplifier when coupled with the wheatstone bridge and the opamp circuitry assisted in not only enhancing differential signals but also filtered out the unnecessary signal harmonics resulting in a $20 \times$ further reduction in the signal noise floor.

8.2 Future Work

8.2.1 Device engineering

The thermal mass of the copper-based PI film devices could be further reduced by

1. Decreasing the thickness of the PI film.

2. Decreasing the thickness of the copper equilibrators through etching mechanisms optimized by controlled etch rates.

3. Exploring different combinations of microfabrication techniques for depositing copper thin film on PI or vice versa in order to minimize the overall thickness of the device.

4. Modifying the square footprint of the copper islands to a less-dense design. For example i) a circular copper island matching the diameter of the outer ring of the heater or ii) two circular concentric copper islands matching the diameters of the RTD and heater connected by copper traces similar in width to their nickel counterparts.

Arrays of differential devices consisting of heater-RTD pairs could be patterned on a single substrate with robust interconnect technology. Simultaneous detection from all the devices in an array has the potential to yield a higher melting signal. Compounding such arrays may lead to a multi-functional detection device capable of sensing multiple biological melting events. The microelectrode array patterns when coupled with commercially available automated droplet dispensing systems will be capable of launching the next generation high throughput microcalorimetric devices.
PCR conformance

The microcalorimetric devices presented in this thesis are fitted with all the components necessary to perform PCR. Coupled with PDMS microfluidics the glass-based microcalorimeters may be used for dynamic dispensing of experimental fluids for running continuous PCR. On the other hand compartmentalizing PCR has been shown to decrease amplification time and increase quantitative assessment of the analyte [116, 110]. Conventional PCR techniques are limited spatially and run slow temperature ramps due to their large thermal masses [179, 196]. Thus this method of ‘dropletizing’ the reaction mixture has significant impact on the speed due to sample containment and throughput due to parallelization. Droplet dispensing by automated systems produce thousands of identical droplets within a single experiment. Manipulating these droplets using electrical fields via on-chip microelectrodes holds promise in reducing the thermal mass of the entire device and speedy response times.

8.2.2 Modular microelectronic circuit design

The PI film device maybe designed stretched out on a light-weight insulating frame integrated with DIP-pin packaging connecting the electrodes such that the entire device can be assembled on a printed circuit board (PCB) along with the modular electronics. Similarly the entire breadboard based electronic circuitry can be integrated into a well-insulated package which can be easily used as a single make-shift signal conditioning unit. Passive devices such as the resistors used in the wheatstone bridge maybe replaced with finer laser-trimmed resistive elements for obtaining cleaner signals. The inclusion of PCB based electronics eliminates the need for using bulky lead wires for making real-world connections. The bridge excitation source could also be integrated with the PCB by designing mixed/RF (radio frequency) analog signal generating circuitry thus eliminating the need for an external function generator. In summary the entire microcalorimetric detection system could be integrated as a single functional unit with a user-friendly interface for the DIP-chip installation and a pin access of its final output from the signal conditioning circuit to couple with an external A/D converter.

This thesis provides a detailed introduction of the critical silicon microfabrication techniques involved in the fabrication of the device. The rationales behind the device design, microelectronic circuitry design and the model systems selected for melting tests have been explained. The objective of the project was to develop a portable, label-free biosensor technology to detect enthalpy changes in biological models suspended in a fluid medium. This novel technology can be adapted for a variety of sensing applications in biomedical devices, the pharmaceutical industry and patient bed-side or field testing. Proof-of-concept in addition to preliminary results
have been presented in this thesis. The proof-of-concept device can be used in the proposed application areas after robust integration, packaging and a multitude of reliability tests to create a commercial Lab-on-a-chip (LOC) device. Collaboration with an industry partner or a strategic “Lab-to-market” development plan could facilitate manufacturing at a commercial scale. Lab environments within universities lack the necessary financial and instrumentation resources thereby limiting progress toward commercialization. Currently the device maybe used for laboratory scale melting experiments for solid or liquid samples in conjunction with the aforementioned electronic instrumentation.
REFERENCES


[9] ASL. AC vs DC.


Le Chatelier. Differential thermal analysis (DTA) and differential scanning calorimetry (DSC). *Physik Chemica*, 1887.


Calorimetry Sciences Corporation. Differential scanning calorimetry (DSC).


Fluke. AC versus DC: The truth.


Tom Hayden and Joel Roop. RTD instrumentation requirements.


National Instruments. RTD, thermistor, thermocouple comparison chart.


Jasco. Thermal denaturation of hen-egg lysozyme with concurrent CD and fluorescence detection.


[152] Labfacility. Thermocouple theory and practice.


[174] EnerCorp Instruments Ltd. Comparision of thermistors, thermocouples and RTD’s.


[232] RdfCorp. Platinum RTD.


[266] Life technologies. Nucleic acid stains.


Appendix A

A.1 Thermal properties

Table A.1: Thermal Properties of Materials Used

<table>
<thead>
<tr>
<th>Material</th>
<th>Thermal conductivity (W/mK)</th>
<th>Density (kg/m³)</th>
<th>Specific heat capacity (J/kgK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>0.0257</td>
<td>1.1</td>
<td>1005</td>
</tr>
<tr>
<td>Glass</td>
<td>1.11</td>
<td>2600</td>
<td>840</td>
</tr>
<tr>
<td>Water</td>
<td>0.58</td>
<td>1000</td>
<td>4187</td>
</tr>
<tr>
<td>Mineral Oil</td>
<td>0.15</td>
<td>800</td>
<td>2130</td>
</tr>
<tr>
<td>Glycerin</td>
<td>0.285</td>
<td>1260</td>
<td>2430</td>
</tr>
<tr>
<td>Polyimide</td>
<td>0.52</td>
<td>1430</td>
<td>1150</td>
</tr>
<tr>
<td>PDMS</td>
<td>0.18</td>
<td>1030</td>
<td>1100</td>
</tr>
<tr>
<td>Nickel</td>
<td>90.7</td>
<td>8900</td>
<td>445</td>
</tr>
<tr>
<td>Copper</td>
<td>401</td>
<td>8900</td>
<td>3900</td>
</tr>
<tr>
<td>Teflon</td>
<td>0.25 - 0.35</td>
<td>2200</td>
<td>1050</td>
</tr>
</tbody>
</table>

A.2 Intermediate Device Generations

A.2.1 4-well glass sandwich device

This generation of devices consisted of two glass substrates integrated with multiple layers of PDMS microfluidics. While glass-based wheatstone bridges formed the sensing unit the glass-based heater performed heating operations. The heater and RTD were both separated by the
PDMS microfluidics between them as seen in Figure A.1. All four RTDs in the bridge were designed to be identical in their base resistance values. While the chambers were 800 µm tall the PDMS mold forming the outlets and inlets to the channels was ≈ 1 cm thick. The thick PDMS layer was an essential part of the device integration as it was used to insert the tygon tubing for sample handling.

The device suffered from high thermal capacitance due to the thermal mass of the various PDMS layers bonded to the device.

Figure A.1: 3D glass-PDMS hybrid device: (a)Micrograph of the glass-PDMS ‘sandwich’ device showing separate inlet and outlet channels for each type of experimental fluid. (b)Cross-sectional view: The PDMS molded for supporting the tubing for the inlet and outlet channels was ≈ 1 cm tall.

A.2.2 2-well PI sandwich device

This generation of devices consisted of two heater-RTD pairs printed on a 12.5 µm thick PI film. The film was embedded within PDMS microfluidics comprising two reaction wells along with separate inlet and outlet channels as seen in Figure A.2. The device was fabricated using PI films without the pre-existing copper cladding. One chamber was meant to station the buffer solution while the other was for the sample. Differential measurement across both RTDs would produce a melting curve in the event of a phase transition. Similar to the the glass sandwich device described above this generation suffered from high thermal capacitance due to the PDMS microfluidics even though the capacitance of the PI substrate was 10× less than that of its glass-based counterparts. In addition copper-less PI films presented various fabrication issues as electrode metallization step led to warping of the polymer film. Such a deformation was a result of the wide difference between the thermal expansion coefficients of nickel and PI.
A.2.3 Double droplet PI devices

This generation of devices contributed to a significant improvement in reducing overall thermal capacitance. PDMS microfluidics were eliminated to accommodate droplet based sensing. The microelectrode design featured a common heater for both RTDs and relied on copper islands for efficient heat transfer to the RTDs. Each RTD footprint was designed to station a 5 µL droplet within its boundary. While one RTD was meant for the buffer droplet the other was...
meant for the sample. Differential measurement of the two RTDs would yield melting curves related to phase transitions within the sample. Eliminating both PDMS microfluidics and the glass substrate reduced the thermal capacitance of the device by several orders in magnitude. However the large footprint of the devices along with their corresponding copper islands led to a high overall thermal mass.