DESIGN AND DEVELOPMENT OF CALORIMETRIC BIOSENSORS USING EXTRAORDINARY OPTICAL TRANSMISSION THROUGH NANOHOLE ARRAYS

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by
Mehmet Ali Sen
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Calorimeter devices and temperature sensors are widely used tools in pharmaceutical, biochemical and clinical diagnostic application areas. The observed energy released during a reaction is a direct measure of the performance of the interaction between tested molecules. This information is important in selecting which compounds should be taken into later stages of drug development process. The main goal in the current calorimeter development research is to reduce the amount of valuable compound used and the experiment time. In the present research, the design and development of a calorimeter utilizing a photonic temperature sensor based on the Extraordinary Optical Transmission (EOT) signals through nanohole arrays (NHAs) was investigated. The calorimeter operation is based on an analytical methodology that relates temperature measurements at discrete locations to evaluate the enthalpy of formation. A 3-D numerical thermal boundary layer analysis demonstrated that surface measurement sensors such as NHA accurately represent the average temperature normal to the flow surface for use in this calculation. The developed calorimeter device is based on a NHA photonic sensor coupled with
a microfluidic system in T-Sensor configuration in which two streams merge into a single channel and mix due to laminar diffusion interface. The width, thickness and length of the flowcell are 500\µm, 100\µm, and 5000\µm, respectively. An interference pattern free optical configuration was developed consisting of a LED light source, polarizer, condenser and a flowcell with a glass-cover and PDMS gasket. Initial experiments confirmed that the EOT phenomenon was observed and EOT vs. temperature calibration agreed with the analytical formulation that the signal gain due to temperature change was dependent on the volumetric expansion coefficient. Reactions of ethanol mixtures/DI water and CaCl$_2$/EDTA compounds were investigated using the developed calorimeter device. The experimental EOT data was compared to the calculated energy release. The results were in agreement with an error less than 5\% and confirm the feasibility of this technology as a calorimetric device.
DEDICATION

To my family
ACKNOWLEDGMENTS

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# TABLE OF CONTENTS

ABSTRACT ..............................................................................................................................i
DEDICATION..........................................................................................................................iii
ACKNOWLEDGMENTS .........................................................................................................iv

1. INTRODUCTION .................................................................................................................1
   1.1. Calorimetry ....................................................................................................................3
      1.1.1. Isothermal Titration Calorimetry .............................................................................9
      1.1.2. Differential Scanning Calorimetry ..........................................................................12
   1.2. Heat and Mass Transfer in Microfluidics and Microchannel Reactors .......................14
   1.3. Surface Plasmon Resonance and Extraordinary Optical Transmission Signal ...........20
   1.4. Optical Detection Methodologies in Microfluidics ......................................................31

2. APPROACH / METHODS .....................................................................................................35
   2.1. Four Port Reaction Chamber ......................................................................................35
      2.1.1. Experimental Setup with PMT sensor .................................................................35
      2.1.2. Flowcell Fabrication ............................................................................................39
   2.2. T-Sensor Type Design ..................................................................................................40
      2.2.1. Theoretical Development - Calorimetric (Energy) Equations in T-Sensor
             Configuration 40
      2.2.2. Experimental Setup with CCD Camera ..............................................................44
2.3. Nanohole Array Sensor Fabrication .......................................................... 48

3. RESULTS AND DISCUSSION ........................................................................... 51
   3.1. EOT Experiments without a Flowcell (Bare Chip) ................................. 51
   3.2. Four Port Reaction Chamber Results .................................................... 55
      3.2.1. EOT versus Temperature Calibration ........................................... 55
      3.2.2. Numerical Analysis ................................................................. 56
      3.2.3. Calorimeter type results ......................................................... 62
   3.3. T-Sensor Results .................................................................................. 71
      3.3.1. Numerical Analysis .................................................................. 71
      3.3.2. Effects of the Light Source and the Flow Cell for NHA Sensors ...... 103
      3.3.3. Effect of Pixel Averaging Size over an NHA site ......................... 119
      3.3.4. Temperature Sensitivity of NHA Sensors – Parametric Analysis ...... 123
      3.3.5. Calorimetric Reactions in Coflow Configuration ............................ 129

4. CONCLUSIONS ............................................................................................... 144

5. RECOMMENDATIONS and FUTURE WORK ............................................ 149

REFERENCES .................................................................................................... 151

APPENDIX A ..................................................................................................... A-1
LIST OF TABLES

Table 3.1: Comparison of the experimental and numerical data. ............................................63
Table 3.2: The constants used for the Arrhenius reaction rate equation. .................................82
Table 3.3: Enthalpy of formation and molecular weight values of the species. ........................82
Table 3.4: List of optical configurations tested. Bare chip means no flowcell was attached on the sensing chip. Tests 9, 10, 11 and 13 were performed with both air (empty) and water in the flowcell channel. ............................................................................................................105
Table 3.5: Physical properties of water and ethanol. ...............................................................131
Table 3.6: Parameters and calculated values for the prepared ethanol mixture in experiments. The volume and calculations are based on the volume for the prepared mixture, not the flowcell volume. .................................................................................................................................131
Table 3.7: Calculated values based on the control volume in the micro-channel. ....................134
Table 3.8: Results of the theoretical and experimental calculations. Superscript exp1 represents 17.13M ethanol mixture/water coflow and superscript exp2 represents 13.70M ethanol mixture/water coflow. .................................................................................................................................141
LIST OF FIGURES

Figure 1.1: Schematic overview of the process for selecting recombinant antibodies for diagnostic use, taken from [2]. ................................................................. 5

Figure 1.2: Thermodynamic profile of the binding of a series of small-molecule inhibitors to HIV protease, taken from [1]. ................................................................. 8

Figure 1.3: ITC setup, taken from [10]. ................................................................................ 9

Figure 1.4: Raw data of a titration experiment of an exothermic reaction releasing heat hence resulting in negative peaks, taken from [10]. ......................................................... 10

Figure 1.5: Integrated raw data output plotted as molar change in enthalpy against molar ratio of A/B, taken from [10]. ........................................................................ 11

Figure 1.6: A protein is denatured in DSC, the peak maximum is the transition temperature and the area under the curve is the enthalpy of the process, taken from [14]. .................. 13

Figure 1.7: T-Sensor configuration. Two liquids containing reactants A and B (with concentrations \([A]_1, [B]_1\)) are in continuous, laminar flow. A reaction, generating product AB, progresses according to interdiffusion of the reactants. .................... 17

Figure 1.8: Schematic of the setup used by Ozdemir and Turhan-Sayan et al. [59]. .............. 26

Figure 1.9: Schematic of the setup used by Krishnan et al. and the temperature sensor, taken from [56]. ........................................................................................................ 27

Figure 1.10: Experimental zero order spectra of a Au film on a quartz substrate \((\varepsilon_2=2.31)\), as a function of refractive index \(\varepsilon_1\). The film thickness is 250 nm, the hole diameter is 200 nm and the lattice constant is \(a_0=600\) nm, taken from [60]. ........................................... 28
Figure 1.11: The relationship of the transmitted light through a nanohole array at different wavelengths as a function of the dielectric constant using the data of Krishnan et al. [60] for gold film on a quartz substrate, taken from [56]. ........................................29

Figure 2.1: Photograph of the experimental setup (Four-port) including the syringe pumps, stage the flowcell assembly and the photomultiplier tube sensor. .............................................36

Figure 2.2: Schematic layout of the device (Top view). ..............................................................................37

Figure 2.3: Device assembly. From left to right: ABS plastic clamp, PDMS layer with the flowcell, glass substrate with gold film and nanohole sensors, thermoelectric heater, ABS plastic base, PMT detector ........................................................................................................37

Figure 2.4: Details of nanohole array chip and SEM image of a nanohole array.................................38

Figure 2.5: Nanohole array chip (gold colored on the back), inlet (1 and 2) and outlet ports (3 and 4) and the PDMS layer..............................................................................................................39

Figure 2.6: Microfluidic reaction chamber manufacturing processes....................................................40

Figure 2.7: Flow geometry of the CFD model. .......................................................................................41

Figure 2.8: Schematic of the optical setup of (a) He-Ne laser light source, (b) LED light source. Red light indicates the optical path.................................................................45

Figure 2.9: Components of the flowcell assembly.................................................................................46

Figure 2.10: Image of the PDMS flowcell, NHA chip with gold surface, copper heat sink........47

Figure 2.11: Cross sectional view of the different flowcell designs, (a) 2mm thick PDMS with 10µm thick flow channel, (b) 100µm PDMS gasket .........................................................48

Figure 2.12: FIB image of a 10 x 10 nanohole array .................................................................49

Figure 2.13: Ion beam dosage time determination........................................................................50
Figure 3.1: (a) CCD image of the NHAs in the center (gray dots), (b) CCD image of the laser intensity when there is no chip in the laser path..............................52

Figure 3.2: (a) Intensity profiles plotted across the NHAs. The spikes correspond to individual NHA sites. (b) Laser intensity profile across the same pixels location when there is no chip.................................................................53

Figure 3.3: A sketch of a single NHA site. Transmission occurs not only through the holes but through the entire rectangular region..................................................54

Figure 3.4: (a) Temperature in the flow cell versus time at the steady state regions. (b) EOT signal versus time at the steady state regions. (c) Cross plot of EOT signal versus temperature.................................................................56

Figure 3.5: Grid and the geometry for the transient analysis.................................................................57

Figure 3.6: Numerical simulation results describing the spatially averaged temperature response of the EOT sensor volume, fluid cell volume and the thermistor volume for the first 200 seconds. The boundary conditions and heat input correspond to the experimental conditions.................................................................58

Figure 3.7: The numerical simulation results describing the spatially averaged temperature response of the EOT sensor volume and the thermistor volume. The boundary conditions and heat input correspond to the experimental conditions [56]. ........59

Figure 3.8: Temperature in the flowcell in the x direction.................................................................60

Figure 3.9: Temperature in the flowcell along the x direction with a copper heat sink between the heater and the sensing chip.................................................................61

Figure 3.10: Experimental setup and thermistor locations.................................................................62
Figure 3.11: Calibration experiment, temperature in different regions plotted with respect to time. Red line represents controlled temperature, black line temperature in the flowcell estimated by the EOT, blue line represents the reservoir temperature.

Figure 3.12: Water injection experiment results. Red Line represents Heater Temperature, Black Line represents Flowcell Temperature measured by EOT signal converted with Figure 3.11 information, Blue Line represents reservoir temperature measured by thermistor (Top Figure).

Figure 3.13: Normalized EOT signals plotted on top of each other with respect to normalized time.

Figure 3.14: Transient response of the thermistor and the EOT signal for the 3rd injection.

Figure 3.15: EOT response of saturated salt solution injections. Each pulse in the plot represents an injection of 4µL saturated salt solution at a rate of 0.1mL/min.

Figure 3.16: Results from salt injections. Response characteristics of the thermistor and heater along with the EOT signal.

Figure 3.17: Comparison of steady state concentration values with theoretically calculated concentration values.

Figure 3.18: Flow geometry of the CFD model.

Figure 3.19: Velocity profile development after the two developed flows merge at the mid-plane, y = d/2 for the case V_z = 0.005 m/s.

Figure 3.20: Comparison of velocity profiles numerically computed by Fluent with the analytical solution [81].
Figure 3.21: Comparison of the temperature field obtained from the numerical simulation (right) at the surface y=0 µm, to the experimentally measured temperature field by Pradere et al. (left) [32]...........................................................................................................................................83

Figure 3.22: Concentration and temperature profiles along the channel width at z=300µm for different grid spacing values. .................................................................................................................................................84

Figure 3.23: Residuals of each calculated value for the simulation with V_z=0.005 m/s. ..............85

Figure 3.24: Normalized temperature profiles for 1M concentration, V_z=0.5m/s, 6M concentration, V_z=0.5m/s.....................................................................................................................................................86

Figure 3.25: (a) Temperature contours for 1M concentration, Q = 25nL/min, (b) corresponding line plot for (a), (c) Temperature contours for 1M concentration, Q = 250nL/min, (d) corresponding line plot for (c), (e) Temperature contours for 6M concentration, Q = 250nL/min, (f) Temperature contours for 6M concentration, Q = 250nL/min, adiabatic case..............................................................................................................................................87

Figure 3.26: Molarity contour plots for (a) 1M concentration, Q = 25nL/s, (b) 6M concentration, Q = 25nL/s, (c) 1M concentration, Q = 250nL/s .................................................................88

Figure 3.27: Development of the temperature profiles at x=w/2 (M=1.0)........................................90

Figure 3.28: Development of the temperature profiles at x=w/2 (M=6.0)........................................90

Figure 3.29: Development of the temperature profiles at x=w/4 (M=6.0).................................91

Figure 3.30: Heat flux values at different x values along the (flow) z direction at the lower and upper surfaces. There is sufficiently low heat flux through the PDMS surface that the traces are nearly indistinguishable for all positions across the channel (dashed lines, top of the plot)..................................................................................................................................93
Figure 3.31: Surface plots of mass fraction of NaCl and the heat loss coefficient \( H(x,z) \) [W/m\(^2\) K] for \( Q = 0.15\mu\text{L/min} \) case. Each dot represents a sensor (shown here 30 \( \mu \text{m} \) spaced, sensor diameter not to scale) taking local information. .......................................................... 95

Figure 3.32: Deviation % of the estimated enthalpy value using Eq. (2.9) from the actual value at different sensor spacing values for different flow rates. .......................................................... 96

Figure 3.33: Deviation % of the estimated enthalpy value using Eq. (2.9) from the actual value at different sensor spacing values for 0.15 \( \mu\text{L/min} \) flow rate using different sensor spacing approaches. ........................................................................................................ 97

Figure 3.34: Plots of Heat loss coefficient, Temperature at \( z=50\mu\text{m} \) and mass fraction of NaCl at \( z=50\mu\text{m} \) and 800\( \mu\text{m} \) as a function of \( z \). Red dots indicate the sensors spaced at 60\( \mu\text{m} \) starting from center. Horizontal red lines present the average values of the curves. ........................................................................................................ 99

Figure 3.35: Deviation % of the estimated enthalpy value using Eq. (2.9) from the actual value at different sensor spacing values for 0.15 \( \mu\text{L/min} \) flow rate using different sensor spacing approaches. Mass fraction calculation was omitted in this calculation. ... 100

Figure 3.36: Plots of simulated noisy (a) temperature and (b) heat loss coefficient signals as a function of \( x \) at \( z = 200 \mu\text{m} \) intervals for \( Q = 0.15 \mu\text{L/min} \) case. ......................... 101

Figure 3.37: Calculated error in the enthalpy estimations of 10 consecutive runs using noisy data obtained from \( Q = 0.15 \mu\text{L/min} \) flow rate. The sensor spacing is 30 \( \mu\text{m} \), and sensors are positioned half the sensor spacing value from the center......................... 101

Figure 3.38: Individual error contribution of each term in Eq. (2.8) at different sensor spacings for \( Q = 0.15 \mu\text{L/min} \) case. ........................................................................................................ 102
Figure 3.39: Cross sectional view of the different flowcell designs, (a) 2mm thick PDMS with 10µm thick flow channel, (b) 100µm PDMS gasket..........................104

Figure 3.40: CCD image of the transmission from a single NHA sensor. The squares represent different averaging regions, blue: 3x3, yellow: 7x7 and red: 13x13......................106

Figure 3.41: CCD images of NHAs with PDMS flow channel (a) with laser illumination, (b) with LED illumination.................................................................107

Figure 3.42: Effect of the temperature on the fringe patterns, T_1<T_2<T_3<T_4 at times t_1, t_2, t_3, t_4 with laser as the light source and with PDMS flow cell. Dashed yellow line highlights the movement of a specific interference pattern.................................109

Figure 3.43: CCD images of NHAs with PDMS flow channel and LED light source (a) with the condenser lens, (b) without the condenser lens, (c) Intensity profiles across the center of the images. Each color in plot (c) corresponds to the intensity variation along the lines with the same color in (a) and (b). Green and red are for the test without the condenser while black and blue are with the condenser in the optical path..................................................................................................110

Figure 3.44: Effect of pressure on the fringe patterns inside the flow channel at isothermal conditions. P_1<P_2<P_3<P_4.................................................................112

Figure 3.45: EOT response during steady and transient temperature for Laser/Glass/Gasket and LED/Glass/Gasket combinations. Temperature was changed from 28°C to 30°C. Dashed ellipse highlights the transient region.........................................................114

Figure 3.46: (a) Raw EOT signal of a temperature calibration experiment for water with the laser, glass cover and PDMS gasket configuration, (b) Controlled unit temperature, (c) EOT vs. temperature cross plot......................................................115
Figure 3.47: (a) Raw EOT signal of a temperature calibration experiment for water and ethanol, (b) Controlled unit temperature, averaged EOT intensity values at different temperatures (c) for water (d) ethanol (e) Normalized intensity value for water and ethanol.

Figure 3.48: CCD image of the transmission from a single NHA sensor. The squares represent different averaging regions, blue: 3x3, yellow: 7x7 and red: 13x13.

Figure 3.49: Temperature calibration results using different averaging regions. (a) to (c) EOT vs. Temperature cross plots, black lines indicate increasing temperature, red lines indicate decreasing temperature. (d) to (f) raw EOT signals at steady temperature region. Each color represent a different set temperature.

Figure 3.50: Temperature calibration results using different averaging regions. (a) to (c) EOT vs. Temperature cross plots, black lines indicate increasing temperature, red lines indicate decreasing temperature. (d) to (f) raw EOT signals at steady temperature region. Each color represent a different set temperature.

Figure 3.51: CCD camera image and the light transmission observed from 15 different NHA designs. SEM images of selected NHAs.

Figure 3.52: (a) Raw EOT signal from 10x10-250nm pitch NHA measured by the CCD camera. (b) Controlled temperature on the copper surface with step changes. (c) Ambient temperature.

Figure 3.53: Average EOT value measured in 12-bit CCD camera units at constant temperature (28.20°C) for 15 NHA sites.

Figure 3.54: Normalized intensity gain in all 15 NHA designs due to a 0.10°C step change from 28.20°C to 28.30°C.
Figure 3.55: Transmission peak wavelength estimation as a function of NHA pitch size using Eq. (1.13).

Figure 3.56: CCD camera image of the reaction channel and the light transmission from the nanohole arrays. Channel walls are highlighted with red lines. The NHA sensors from 1 to 38 are shown with arrows.

Figure 3.57: Sketch showing three stages of the experiment. EOT was measured during 1. injection of the same reactant A, 2. coflow of A and B, 3. during the mass and thermal equilibrium state.

Figure 3.58: Enthalpy of dilution of ethanol-water mixtures.

Figure 3.59: Mixing region and the control volumes used for calculations.

Figure 3.60: Raw EOT signal during pure ethanol, water dilution plotted with respect to time at $z_1=900\mu$m. An average intensity was calculated by averaging the raw signal over time indicated with green and red circles.

Figure 3.61: Raw EOT signal during pure ethanol, water dilution plotted with respect to time at $z_1=2200\mu$m. An average intensity was calculated by averaging the raw signal over time indicated with green and red circles.

Figure 3.62: Raw EOT signal at steady state during water flow in both inlets, $t_1$, indicated as black and Raw EOT signal during coflow steady state, $t_2$, red lines. Both $z$ locations were reported.

Figure 3.63: Intensity difference across channel width (x-direction) at $z_1=900\mu$m for three different concentrations of ethanol-water mixture. Dashed lines represent scaled diffusion only model.
Figure 3.64: Intensity difference across channel width (x-direction) at $z_2=2200\mu m$ for three different concentrations of ethanol-water mixture. Dashed lines represent scaled diffusion only model.

Figure 3.65: Intensity difference across channel width (x-direction) at $z_1=900\mu m$ $z_2=2200\mu m$ for coflow reaction of 0.1mM EDTA and CaCl$_2$ solutions in MES buffer.

Figure 3.66: Repeat of the experiment. Intensity difference across channel width (x-direction) at $z_1=900\mu m$ $z_2=2200\mu m$ for coflow reaction of 0.1mM EDTA and CaCl$_2$ solutions in MES buffer.
## NOMENCLATURE

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>area (m(^2))</td>
</tr>
<tr>
<td>(A_{\text{arrhenius}})</td>
<td>pre-exponential factor</td>
</tr>
<tr>
<td>AB</td>
<td>product</td>
</tr>
<tr>
<td>D</td>
<td>mass diffusion coefficient (m(^2)/s)</td>
</tr>
<tr>
<td>(D_{\text{hyd}})</td>
<td>hydraulic diameter (m)</td>
</tr>
<tr>
<td>(E_a)</td>
<td>activation energy for the reaction (J/(kmol))</td>
</tr>
<tr>
<td>(\dot{E})</td>
<td>energy (W)</td>
</tr>
<tr>
<td>EOT</td>
<td>Extraordinary Optical Transmission</td>
</tr>
<tr>
<td>H</td>
<td>heat transfer coefficient on the chip surface (W/(m(^2).K))</td>
</tr>
<tr>
<td>(\Delta H)</td>
<td>enthalpy (J/mol)</td>
</tr>
<tr>
<td>I</td>
<td>Intensity (CCD camera units, 0-4095)</td>
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<tr>
<td>J</td>
<td>diffusion flux (mol/(m(^2).s))</td>
</tr>
<tr>
<td>Kn</td>
<td>Knudsen number</td>
</tr>
<tr>
<td>L</td>
<td>channel length in z-direction (m)</td>
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<td>(L_c)</td>
<td>characteristic length (m)</td>
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<tr>
<td>(M)</td>
<td>molar mass (kg/mol)</td>
</tr>
<tr>
<td>NHA</td>
<td>Nanohole Array</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>(\text{Pe}_M)</td>
<td>Peclet number for mass diffusion</td>
</tr>
<tr>
<td>(\text{Pe}_T)</td>
<td>Peclet number for thermal diffusion</td>
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<tr>
<td>Q</td>
<td>volumetric flow rate (nL/s)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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<tr>
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<td>-------------</td>
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<tr>
<td>$R_{\text{gas}}$</td>
<td>universal gas constant (J/(K·mol))</td>
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<tr>
<td>$R$</td>
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<tr>
<td>$\dot{R}$</td>
<td>Arrhenius molar rate of creation/destruction of species</td>
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</tr>
<tr>
<td>SPR</td>
<td>Surface Plasmon Resonance</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature (K)</td>
</tr>
<tr>
<td>$V$</td>
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<tr>
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<tr>
<td>$X$</td>
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<tr>
<td>$c_p$</td>
<td>specific heat at constant pressure (J/(kg·K))</td>
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<tr>
<td>$d$</td>
<td>channel thickness in y-direction (m)</td>
</tr>
<tr>
<td>$e$</td>
<td>enthalpy (J/kg)</td>
</tr>
<tr>
<td>$g$</td>
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</tr>
<tr>
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<td>heat transfer coefficient (W/(m²·K))</td>
</tr>
<tr>
<td>$k$</td>
<td>thermal conductivity (W/(m·K))</td>
</tr>
<tr>
<td>$k_f$</td>
<td>forward reaction rate constant</td>
</tr>
<tr>
<td>$l$</td>
<td>mean free path (m)</td>
</tr>
<tr>
<td>$\dot{m}$</td>
<td>mass flow rate (kg/s)</td>
</tr>
<tr>
<td>$n$</td>
<td>stoichiometric coefficient</td>
</tr>
<tr>
<td>$p$</td>
<td>static pressure (Pa)</td>
</tr>
<tr>
<td>$q''$</td>
<td>heat flux (W/m²)</td>
</tr>
<tr>
<td>$w$</td>
<td>channel width in x-direction (m)</td>
</tr>
</tbody>
</table>
x coordinate in the channel width direction
y coordinate normal the flow (chip) surface
z coordinate in the flow direction

Greek symbols:

\( \nabla \) differential operator
\( \alpha \) thermal diffusivity (m\(^2\)/s)
\( \nu \) kinematic viscosity (m\(^2\)/s)
\( \rho \) density (kg/m\(^3\))
\( \tau \) shear stress (Pa)

Subscripts

a activation
amb ambient
ent entrance
gen generated
hyd hydrodynamic
set set value
xy plane normal to z-direction
xz plane normal to y direction
yz plane normal to x-direction
Superscripts

\text{T} \quad \text{transpose}

\rightarrow \quad \text{vector}

\_\_ \quad \text{averaging in one direction}

\_\_\_ \quad \text{averaging in two directions}
1. INTRODUCTION

Calorimetry is an essential tool in research and development areas including pharmaceutical industry and, drug development research. Calorimetry provides information for decision making in lead discovery and optimization [1]. Unlike the present high-throughput screening methods used by the industry, like the affinity sensors, calorimetric analysis can provide very detailed information on the interaction between molecules. Calorimetry provides detailed thermodynamic information including enthalpy of reaction term $\Delta H$ and binding constant $K_b$. These terms can be used to calculate entropy change $\Delta S$, rate of disassociation constant $K_d$ and, change in free energy $\Delta G$. Measurement of enthalpy of reaction, $\Delta H$, gives information regarding the interaction between the reactants (drug candidates). Measurement of entropy change, $\Delta S$, can be used to calculate the irreversibility during the binding between the molecules. $\Delta H$ represents the bonding energy between the molecules. The product of $\Delta S$ times temperature represents irreversible (not utilized) energy. Hence, optimization for a compound can be done by aiming at decreasing irreversibility ($\Delta S$) and increasing the contribution of enthalpy of reaction term ($\Delta H$). In the current technology, initial high throughput screening and the first candidate drug selection is performed by affinity analysis. Next, candidates are analyzed further by the two currently available calorimetry techniques: Differential Scanning Calorimetry and Isothermal Scanning Calorimetry. These techniques are capable of measuring thermochemical properties ($\Delta H, K_b, \Delta S, \Delta G$) of a reaction.

Current research and instrument development efforts in calorimetry concentrates on getting calorimetric information using less compound and reducing experiment time by introducing new sensing technologies combined with microfluidic reactant delivery approaches.
The current approaches in literature will be discussed in the following sections. The primary outcome of our research was to demonstrate that Nanohole Array Sensing technology can be used as a new sensing technology for calorimeter instrument research and development. Our experimental results demonstrate that this approach was feasible and that it is more sensitive than the current technology.

Surface Plasmon Resonance (SPR) and Extraordinary Optical Transmission (EOT) phenomena was utilized as a temperature sensor leading to a very sensitive, fast response and multiplexed (capability of observing more than one reaction at a time) calorimeter device. Nanohole arrays in a conductive film deposited on a dielectric surface were used as the sensing medium. Using Nanohole array temperature sensing technology would result in minimization of the valuable reactants used and shorter run times than the current technology.

Our research involves the design and fabrication of nanohole array chip sensor, capable of extracting temperature information of a reaction region. Chip design includes the positioning and distribution of the nanohole array sensors on the flow surface to extract necessary information from correct places. On the instrumental level, hydrodynamic, thermal and optical characteristics of the assembly (flowcell, sensing chip, light source) also need to be investigated to understand system characteristics. This investigation is especially important for optical systems where the device assembly consists of several reflective assemblies. This research is also concentrated on the post-processing of the data obtained by the nanohole array sensors. This includes analytical relation of temperature measurements at discrete NHA sensing locations to the enthalpy of reaction.
The current study focuses on extension of this technology to a temperature sensor in a microchannel reactor. The research includes thermal and fluidic design of a microfluidic system ensuring accurate reactant delivery and predictable thermal behavior characteristics. A numerical model was used to obtain detailed information on the thermal boundary characteristics of the system. Thermal boundary analysis is important since EOT signals are affected in a small distance normal to the chip surface. By analyzing and understanding the thermochemical heat transfer processes in the microchannel, one can obtain valuable information such as reaction enthalpy of a specific reaction ($\Delta H$) and binding equilibrium constant ($K_b$) leading to entropy generated during the reaction ($\Delta S$). Calorimetric information is vital to the pharmaceutical and drug development industries for the decision making process [1] and could be obtained from microchannel reactors which decrease the mass of the samples used significantly.

The outline for this section is as follows: The calorimetry concept is discussed and two widely used calorimetric techniques are explained. Literature related to heat and mass transfer in microchannels is discussed. The Surface Plasmon effect is described following the EOT literature. Applications in the literature utilizing EOT signal are presented and EOT signal and temperature sensor characteristics are discussed in detail. Finally, optical detection technologies and optical characteristics of microfluidic systems are discussed.

1.1. Calorimetry

Information acquired from molecular interactions is critical in many research areas, including biophysics, life sciences and food research. High-throughput screening (HTS) methods are used to analyze the interaction between a potential drug candidate (antibody) and a target protein. Current HTS techniques are capable of analyzing large numbers of drug candidates however these techniques usually give limited, binary (hit/ no hit) information. Hence, HTS
techniques are used in the initial stages of the drug development processes. An example process chart for a drug development process is shown in Figure 1.1 [2]. This figure shows the number of target proteins tested at each step of the process. Clone in Figure 1.1 refers to the physical piece of DNA segment that has been localized to a particular region of a chromosome. In the beginning of a drug development process, the initial number of clones to be tested is on the order of $10^9$. Current methodology followed in the industry is to use secondary screening methods after reducing as many initial number of drug candidates as possible ($10^1$) using binary selection methods.

Current research in the instrument development for drug development industry focuses on technologies capable of multiplexing and which can give detailed information regarding interaction kinetics in shorter run times. In addition, increase in the sensitivity of the instruments can lead to the minimization of the valuable protein consumption. Considering the amount of clones used in each stage of the candidate drug selection process, reduction in the protein consumption is very important. This reduction can be achieved by increasing the sensitivity of the sensing technologies.

Current methodology in the industry involves using affinity/binding sensors as the secondary screening tool. Binding sensors utilizing Surface Plasmon Resonance (SPR) technology are capable of multiplexed sensing with high sensitivity. Biacore™ label-free interaction analysis by GE Healthcare currently uses this technology and a good example for how new sensitive, multiplexed technologies can be utilized in this area. This approach can give quantitative information regarding binding kinetics based on the association and disassociation curves. However, further analyses are usually required on different platforms to reduce the number of the drug candidates. For these analyses, instruments which can give detailed
information for the interaction kinetics are required. Calorimeter instruments are such devices but in the current technology they lack multiplexing capability.

![Diagram of antibody selection process](image)

**Figure 1.1:** Schematic overview of the process for selecting recombinant antibodies for diagnostic use, taken from [2].

Calorimetry provides detailed and quantitative information for decision making in lead discovery and optimization [1]. Characterization of the thermodynamics of binding interactions is important in improving our understanding of biomolecular recognition and forms and essential part of the rational drug design process [3]. Thermodynamic studies of molecular interactions
provide a wealth of information that is not only of fundamental scientific interest but also of immense practical utility in terms of biotechnology, medicine and drug design [4].

For a molecular interaction, calorimetry provides quantitative information of the change in free energy (Gibb’s free energy, \(\Delta G\)) and its component quantities: equilibrium binding constant (\(K_b\)), the change in enthalpy (\(\Delta H\)), and change in entropy (\(\Delta S\)). The \(\Delta G\) value provides quantification of the affinity of binding between two interacting molecules [1].

Most calorimetric experiments start with experimental determination of the equilibrium binding constant, \(K_b\) [4]. Using Eq. (1.1), \(\Delta G\) value can be estimated.

\[
\Delta G = -RT \ln K_b
\]  (1.1)

where, \(T\) is the absolute temperature in Kelvin units and \(R\) is the gas constant. The equilibrium binding constant \(K_b\) is the reciprocal of the equilibrium disassociation constant \(K_d\),

\[
K_d = \frac{1}{K_b}
\]  (1.2)

In addition, calorimetric experiments provide a measure of the heat energy (\(\Delta H\)) associated with forming a macromolecular complex at a given temperature. This enthalpic term is a direct measure of the net change in the number and/or strength of the non-covalent bonds on going from the free to the bound state.

The Gibbs free energy of a reaction at any temperature can be described as:

\[
\Delta G(T) = \Delta H(T_R) + \int_{T_R}^{T} \Delta c_p \, dT - T \Delta S(T_R) - T \int_{T_R}^{T} \Delta c_p \, d\ln T
\]  (1.3)
Eq. (1.3) can be reduced to Eq. (1.4) by setting $T = T_R$, at constant experiment temperature. After obtaining $\Delta G$ and $\Delta H$ values, remaining term contributing to the free energy (product of entropy and temperature) at a certain temperature can be calculated.

$$\Delta G = \Delta H - T \Delta S$$  \hspace{1cm} (1.4)

Unlike the present high-throughput screening methods used by the industry, such as the affinity sensors, calorimetric analysis can provide very detailed information on the interaction between molecules. Measurement of enthalpy of reaction, $\Delta H$, gives information regarding the interaction between the reactants (drug candidates). Measurement of entropy change, $\Delta S$, can be used to calculate the irreversibility during the binding between the molecules. $\Delta H$ represents the bonding energy between the molecules. $\Delta S$ and temperature product represents irreversible (not utilized) energy. Hence, optimization for a compound can be done by aiming at decreasing irreversibility ($\Delta S$) and increasing the contribution of enthalpy of reaction term ($\Delta H$). Ladbury et al. [1] illustrates the utilization of these terms for optimization in drug research in Figure 1.2.

Figure 1.2 shows the enthalpic optimization process. Starting on the left side, the x axis represents the timeline and the next generation drugs. As seen from the figure, as one moves from left to right (i.e. as the time evolves), enthalpy contribution to the total available Gibb’s free energy is the highest hence the entropic contribution is less. This ensures that most of the energy goes to the atomic/molecular bonding rather than lost in the irreversibility of the chemical reaction. Another conclusion is that Gibbs’ free is relatively constant and that entropy is the term which is usually modified because it is easier rather than the enthalpy. Each name on the x axis corresponds to a FDA approved drug introduced to the market. It is important to note that the span of the x axis is 11 years and if drugs were selected based on thermodynamic data the last “most effective” drug introduced in 2006 could have been discovered earlier.
There are two widely accepted and industrially used calorimetric techniques, isothermal titration calorimetry (ITC) and Differential Scanning Calorimetry (DSC). Other than these techniques, there are non-traditional calorimetric methods proposed by researchers. These methods are summarized in Microchannel Reactors section.

ITC and DSC can be considered as complementary techniques. Order-disorder transitions, protein unfolding of nucleic acid melting can be studied using DSC. DSC is also used for quality testing and research tool in the materials research field (Glass transition, crystallization, oxidation stability). Ligand-macromolecule or macromolecule-macromolecule binding interactions, protein-drug, drug-DNA, protein-DNA interactions, the kinetics and thermodynamics of enzyme catalyzed reactions can be examined using ITC [4].
1.1.1. *Isothermal Titration Calorimetry*

Application areas and the working principles of the ITC technique are well documented in literature [1, 3-8]. ITC is the most direct method to measure the energy change on formation of a complex at constant temperature [5]. In a typical ITC instrument the change in enthalpy on binding is measured in a small reaction cell (approximate volume of 0.2 mL to 1.5 mL). Two identical cells, one for interaction (the sample cell) and one to act as a reference are located in a jacket, which is kept at a temperature below that at which the experiment is to be conducted [9]. The setup is illustrated in Figure 1.3.

![Figure 1.3: ITC setup, taken from [10].](image-url)
The reference cell contains buffer or water and is sealed. The two cells are heated to experimental temperature. The power applied to the cell to maintain constant temperature is measured and provides the baseline value at thermal equilibrium and their temperature is monitored by a thermocouple device [9]. The experiment is performed by titrating one binding partner (titrant A) into a solution containing the other binding partner (titrand B) in the sample cell of the device [5]. After each addition of small aliquot of A, the heat released or absorbed in the sample cell is measured with respect to a reference cell filled with buffer (or water). The heat change is expressed as the electrical power (J s\(^{-1}\)) required to maintain a constant small temperature difference between the sample cell and the reference cell, which are both placed in an adiabatic jacket.

![Raw data of a titration experiment of an exothermic reaction releasing heat hence resulting in negative peaks, taken from [10].](image)

Figure 1.4: Raw data of a titration experiment of an exothermic reaction releasing heat hence resulting in negative peaks, taken from [10].
The contents of the sample cell are stirred to effect rapid mixing of the reactants. The amount of titrand required per experiment depends on the magnitude of the heat change; 10-100 nmol of protein are typical [5]. Figure 1.4 shows the raw data of an ITC experiment. Each peak corresponds to the energy released on addition of an aliquot of ligand to the receptor. Integration of each peak with respect to time determines the energy change which equals the heat transfer from the sample cell at each injection.

Since the total concentration of the components of the interaction at any time during the titration can be calculated, the integrated heat transfer can be plotted with respect to molar ratio of A and B. For a simple interaction with a stoichiometry of one independent binding site under appropriate conditions this gives a sigmoidal isotherm as shown in Figure 1.5.

![Graph showing integrated raw data output plotted as molar change in enthalpy against molar ratio of A/B](image)

**Figure 1.5:** Integrated raw data output plotted as molar change in enthalpy against molar ratio of A/B, taken from [10].
To obtain a sigmoidal curve as in Figure 1.5, the product of stoichiometry of the interaction, n, and equilibrium binding constant, nK_b must be between 10 and 100 [9], where n is the stoichiometry of reaction. This value governs the shape of the sigmoidal curve illustrated in Figure 1.5. Low nK_b values result in straight lines and for high nK_b values this curve becomes near vertical.

For an injection, the heat removed or energy released can be written as,

\[ Q = [B_{tot}]V_0(F_2 - F_1)\Delta H \] (1.5)

Where, V_0 is the volume of the cell and F is the fraction of B bound. F can be written as,

\[ F = [AB]/[B_{tot}] \] (1.6)

And,

\[ K_b = [AB]/([A][B]) \] (1.7)

Combining Eqs. (1.5), (1.6) and (1.7) gives,

\[ [AB]^2 - \left( [A_{tot}] + [B_{tot}] + \frac{1}{K_b} \right) [AB] + [A_{tot}][B_{tot}] = 0 \] (1.8)

Using the sigmoidal curve in Figure 1.5 and Eq. (1.8) a curve fitting methodology can be applied to estimate the terms K_b and \( \Delta H \). These terms are used to calculate remaining thermodynamic terms mentioned above.

1.1.2. Differential Scanning Calorimetry

The theory behind DSC and the application areas have been documented by several researchers [4-5, 11-13]. DSC is the most direct experimental technique to resolve the energetic of conformational transitions of biological macromolecules [5]. DSC is routinely used to study an entire range of biomolecular interactions, protein stability, protein denaturation, lipid phase
transitions, surfactant micellization, nucleic acid ‘melts’ and stability of liquid biopharmaceuticals as well as less defined cellular systems [4]. This technique is also used in material science area to study glass transition, crystallization, and melting properties of materials.

Figure 1.6: A protein is denatured in DSC, the peak maximum is the transition temperature and the area under the curve is the enthalpy of the process, taken from [14].

A DSC instrument contains two cells suspended in an adiabatic jacket and connected by various heating and temperature / power sensing circuits [4]. During a normal experimental set-up the reference cell is filled with buffer and the sample cell is filled with identical buffer plus macromolecule (protein, nucleic acid, lipid, cell volume 0.5 mL to 1 mL) and the temperature is increased quasi-adiabatically, typically 0.5 to 1.5 K min\(^{-1}\) [5]. Since the heat capacities of the solution in the sample cell and the solvent in the reference cell differ, a certain amount of electrical power is required to zero the temperature difference between two cells. The power
difference (J s⁻¹), after normalization by the scanning rate (K s⁻¹), is a direct measure of the heat capacity difference between the solution and the solvent [5]. In addition, any energy released due to folding, salvation or instability will be detected.

Experimentally obtained heat capacity can be used to calculate calorimetric transition enthalpy, ΔH,

\[ \Delta H = \int C_p \, dT \]  \hspace{1cm} (1.9)

The integrated area is also illustrated in Figure 1.6 [14]. Using the experimentally obtained temperature profile and the heat capacity curve, entropy for change of transition can be determined,

\[ \Delta S = \int \frac{C_p}{T} \, dT \]  \hspace{1cm} (1.10)

The temperature at which excess heat capacity is at a maximum defines the transition temperature, \( T_m \). Using \( \Delta H \) and \( \Delta S \), \( \Delta G \) can be calculated at the experimental temperature.

**1.2. Heat and Mass Transfer in Microfluidics and Microchannel Reactors**

Microfluidics has been widely used in the areas ranging from biology, chemistry, physics and engineering to reduce sample volumes, increase reaction speed, increase measurement sensitivity, and shrink device dimensions. A particular area where the microscale is advantageous is calorimetry. By analyzing and understanding the thermochemical heat transfer processes in the microchannels, one can obtain valuable information such as reaction enthalpy, entropy, Gibbs free energy, binding, and equilibrium constants. Calorimetric information is vital to the pharmaceutical and drug development industries for the decision making process [1].
Sample throughput and the amount of sample needed to execute a calorimetry experiment could be significantly decreased using microchannel reactors.

Calorimetry requires control of both mass and heat transport. One example of a microfluidic device in which mass diffusion is the main mixing mechanism is the T-sensor introduced by Yager et al. [15] (see Figure 1.7). The T-sensor takes advantage of the low Reynolds number flow conditions in microfluidic channels. The interdiffusion and resulting chemical interaction of components from two or more input fluid streams are monitored optically by measuring the fluorescence intensity, which is dependent on the concentration of the reactants and products (see Figure 1.7 [15]. In Figure 1.7 the plume of reacted compound, AB, is illustrated. At any plane perpendicular to the flow direction, the concentration value is at $[A]_1$ (concentration of compound A) and starts to decrease in the vicinity of the reaction interface. In the reaction zone, all three species coexist, and their concentration values are space dependent. In the vicinity of the lower plume boundary layer, concentration of compound A reduces to zero and the remaining fluid is at concentration value $[B]_1$ (concentration of compound B). A theoretical analysis of the molecular diffusion process in T-sensors has been published by Kamholz and Yager [16]. Using the same sensor setup, Salmon et al. [17], [18], studied the diffusion dynamics and the rate constants of the reactions. Recently, an experimental and computational study was performed using surface plasmon resonance detection technology to study the diffusion kinetics of a small molecule analyte into a parallel-flowing stream containing an antibody in a T-sensor [19], [20].

Taylor dispersion which occurs in high shear flow can affect the diffusion process has been investigated for the T-Sensor configuration. Beard [21] investigated the Taylor dispersion in the T-Sensor geometry introduced by Kamholz et al. [15]. Taylor [22] developed an
expression for solute dispersion along the axial direction (z-direction) in laminar pipe flow, the Taylor dispersion, under the assumption that radial concentration profiles are dissipated rapidly relative to the time scale for advective transport.

Aris [23] extended the expression developed by Taylor [22] to more general cases. He investigated analytical solutions for cases such as Taylor dispersion in rectangular channels, special initial distribution of the solute and turbulent flow in circular cross section tubing.

Ajdari et al. [24] investigated diffusion in shallow microchannels. Their most important conclusion is that hydrodynamic dispersion in shallow microchannels is in most cases controlled by the width of the cross section rather than by the much thinner height of the channel. Their study analyzed transient regimes in Taylor dispersion and provided analytical relations for shallow microfluidic channel shapes. The transient analysis is important for plug and droplet based injections where a plug of compound travels in the channel in contact with the buffer solution. These types of injections are frequently used in microfluidics and dispersion in axial direction is significant in these cases. The transient analysis is necessary in the plug injection configuration because dispersion within the plug boundaries evolves as the plug travels across the channel. These regimes are investigated in detail by Ajdari et al. [24].

Beard’s investigation [21] considered a steady state system and fully developed velocity and concentration profiles since T-Sensor measurements by Kamholz were based on a continuous flow of two reactants until a steady state was reached (not plug or droplet type injection). Beard questioned the assumption made by Kamholz et al. [15] about neglecting axial mass diffusion since there is Taylor dispersion in this direction. Beard provided a modified diffusion term, which includes Taylor dispersion in the flow direction, for the two dimensional
mass diffusion equation used in T-Sensor by Kamholz et al. [15]. The constant in the modified diffusion term by Beard was corrected by Dorfman et al. [25]. Beard [26] numerically showed that the mass diffusion width narrows down at high speed flows (0.1m/s or higher in his study) when the modified diffusion coefficient used. This effect is minute compared to the transient cases [26]. For slower speeds, the solution with the modified diffusion term did not significantly differ from the solution used by Kamholz et al. [15-16].

In this research, the continuous flow approach was used rather than plug and droplet type injection. Our numerical and experimental analyses were both performed for steady state conditions. In addition, the axial diffusion was included in the 3-D numerical simulation which is presented in the Results section.

![T-Sensor configuration](image)

**Figure 1.7:** T-Sensor configuration. Two liquids containing reactants A and B (with concentrations $[A]_1$, $[B]_1$) are in continuous, laminar flow. A reaction, generating product AB, progresses according to interdiffusion of the reactants.

There have been many investigations on the mass transport properties of microreactors in the literature. However, the heat transport has received less attention. Further information on
reaction kinetics can be obtained by studying the heat transfer processes taking place in the T-sensor configuration. Though the governing equations appear similar, heat transport, unlike mass transport, involves flux across multiple boundaries, making it more complicated to solve. There is heat flow across the boundaries in practical applications while there is no mass flux across these boundaries of the flow channel. Another simplification which is often permissible in the mass transport problem is that diffusive transport is negligible in the flow direction because the mass transport Peclet Number, $Pe_M$, is large. The Peclet Number ($Pe$) is the ratio of the convection term to the mass or thermal diffusion term and defined as $Pe_M = \frac{D_{hyd} V_z}{D}$ for mass diffusion and $Pe_T = \frac{D_{hyd} V_z}{\alpha}$ for thermal diffusion. $D$ is the diffusion coefficient, $D_{hyd}$ is the hydraulic diameter of the flow channel ($4A_{xy}$/Perimeter), $\alpha$ is the thermal diffusivity defined as $\alpha = k/\left(\rho \cdot c_p\right)$. Due to the small values of binary mass diffusion coefficients, the $Pe_M$ number in the flow direction is usually very large. However, for the energy equation at low Reynolds number flow, and the contribution of the convective and diffusive terms must be included in the analysis because the thermal Peclet Number, $Pe_T$, is small.

Heat transfer in rectangular microchannels has been addressed by many researchers. Tunc et al. [27] presented an analytical solution for the convective heat transfer with no source term in 2-D rectangular microchannels for the fully developed flow and for a range of different Knudsen numbers with constant heat flux across the boundaries. Knudsen number is defined as $Kn = l/L_c$, where $l$ is the mean free path and $L_c$ is the characteristic length. Since our simulation deals with room temperature liquids at low Re number flows, Kn number for our case is in the hydrodynamic regime and no slip boundary condition is valid. Ambatipudi et al. [28] numerically studied the conjugate heat transfer between fluid in microchannels and a silicon wafer at high Reynolds numbers in a 3-D configuration to optimize cooling of the solid region.
The conjugate heat transfer between the liquid and the PDMS flowchannel was insignificant compared to the interaction between the fluid and the wafer surface due to the low conductivity of PDMS ($k_{PDMS}=0.15\text{W/mK}$) compared to the wafer surface. Toh et al. [29] and Kroeker et al. [30] analyzed microchannels for convective cooling purposes in 3-D domains. The main motivation for their work was cooling of electronic devices and therefore it was directed toward convective heat transport. The heat generation source term is not included in the energy equation in these previous studies.

In recent years, the analysis of heat transfer processes in microreactors has been used to extract thermochemical information. Zhang et al. [31] used microthermophiles in a microfluidic reaction chamber to monitor the temperature change during a reaction. Pradere et al. [32] used infrared thermography to measure the temperature field of nonreacting and reacting flows and proposed a field processing method to retrieve the source term due to the reaction. Fudym et al. [33] performed a detailed two-dimensional analytical study of the heat transfer between multilayered systems. This analytical model is then applied to temperature fields obtained by infrared thermography from the work by Pradere et al. [32]. In both approaches by Pradere et al. [32] and Fudym et al. [33], the temperature field used in the calculations represents temperature averaged along the depth of the microchannel (and also some portion of the encapsulating materials) and it is assumed that this average does not differ from the average flow field temperature. This is a result of using single surface measurements such as infrared thermography. Hany et al. [34] used thermopiles to directly measure the global heat flux dissipated during the chemical reaction in a microfluidic chip for coflow and droplet flow configurations. Recently, Lee et al. [35] presented an approach to an enclosed microfluidic calorimeter using thermophiles. In this work, they combined vacuum encapsulation with
thermopile sensing and microfluidic injection circuitry. The microfluidic injection allowed for injection of nanoliter volumes into a 3.5nL reaction chamber. The vacuum enclosure design helped to create an extremely low thermal conductance of the reaction chamber, 16µW/K. This enabled a power resolution of 4.2nW for energy measurements in the 10nJ range. They suggested that improvements can be made with better selection of thermopiles.

1.3. Surface Plasmon Resonance and Extraordinary Optical Transmission

Signal

There has been intensive research in the area of Surface Plasmon Resonance and Extraordinary Transmission. Numerical, theoretical and experimental approaches are available in the literature. Recently, an extensive literature review of the SPR and EOT has been conducted by Coe et al. [36].

Collective excitation of surface electrons in metals induced by lights is called Surface Plasmons and has been reported in the literature as early as 1957 [37]. Surface plasmons are essentially light trapped at a metal’s surface by its interaction with the metal’s conducting electrons, which act like plasma [36]. The incident light excites an oscillation of the electron clouds that is localized on the metal surface. Such excitations can be transferred to similar, adjacent structures if they are sufficiently close. Periodic arrays of coupled particles enable excitations to propagate along the arrays like ripples on a pond [36].

Barnes et al. [38] discussed how the propagation of the SP waves can be manipulated using wavelength scale periodic structures to build new photonic structures. Possible uses of miniaturized photonic circuits based on SPR such as Bragg reflectors, hole arrays to alternate SPR waves and generation of light using SPR based photonic technologies were discussed [38].
Light transmission through a “small hole” in an infinitely thin perfectly metal screen has been analyzed by Bethe in 1944 [39]. Bethe’s theory suggests that the transmission, normalized to the area of the hole, scales as $(d/\lambda)^4$, where $d$ is the hole diameter and $\lambda$ is the wavelength.

Ebbesen et al. [40] utilized the interaction of incident light and surface plasmons in optically thick metal surface with subwavelength hole arrays and showed that light can be transmitted extremely efficiently, resulting in the EOT signal. Ebbesen et al. [40] defined the absolute transmission efficiency by dividing the fraction of light transmitted by the fraction of surface area occupied by the holes. Transmission efficiency was defined in Eqn. (1.11).

$$\frac{(EOT \text{ Transmission}/Bethe's \text{ Prediction})}{N_{holes} \cdot Area_{hole}/Area_{NHA}} \quad (1.11)$$

They reported that the absolute transmission efficiency can be greater than unity and need not be on the order of $10^{-3}$ (based on the Bethe’s $(d/\lambda)^4$ relation for 150 nm apertures and incident light with a wavelength of 632 nm). More than twice as much light is transmitted as impinges directly on the hole area. An extensive literature review of the surface plasmon resonance and EOT was conducted by Coe et al. [36]. This analysis was explained further in the Results section using our experimental setup.

Degiron et al. [41] analyzed the dependence of EOT signals on the aperture shape. They measured the transmitted intensity at various angles from a rectangular aperture on Ag metal films (wavelength ranging from 400nm to 900nm). Their results suggested that intensity shows an angular dependence, being maximum normal to the surface.

Degiron et al. also [42] studied the effect of hole depth (conductive film thickness) on the transmission of structures having same array period and hole diameter (300 nm) on Ag metal
films. They performed wavelength spectra transmission analysis and their results showed increasing transmission as the hole depth decreases. They relate the transmission intensity to the hole depth, \( h \), peak wavelength, \( \lambda_p \), and hole diameter \( d \) as follows where \( C \) is a proportionality constant:

\[
l(h, \lambda_p, d) = C \cdot \exp \left[ -\frac{4\pi h}{\lambda_p} \sqrt{\left(\frac{\lambda_p}{1.7d}\right)^2 - 1} \right]
\]  

(1.12)

Koerkamp et al. [43] performed a similar research by analyzing wavelength spectra transmission light intensity through periodic circular and rectangular subwavelength apertures on gold films. Three different shapes were milled on a 200 nm gold film, with an array period of 425 nm: a circular hole array with a diameter of 190 nm, an array of rectangular holes of 75 x 225 nm\(^2\) and an array of rectangular holes of 150 x 225 nm\(^2\). Their experimental results suggest that transmittance is higher by an order of magnitude in rectangular hole shapes compared to circular hole arrays.

Van der Molen et al. [44] studied the hole size dependence in rectangular subwavelength apertures. Rectangular hole arrays with a period of 425 nm are milled on a 200 nm Au film. The width of the square holes is varied in the range of 150 to 290 nm. Their results indicate that an increase in hole width results in a higher transmissivity and broader peaks.

Bravo-Abad et al. [45] theoretically investigated the effect of array shapes on transmission on a perfect conductor metal. Their analysis is based on the comparison between the transmission properties of finite Penrose lattices exhibiting rotational symmetry with rectangular periodic arrays and random distributed arrays. The number of holes in all lattices was constant and equal to 1506. The transmitted light was measured at different wavelengths. The
result showed that random distributed hole array shows similar trend with a single hole array, which is smooth decreasing function of the wavelength. For the ordered rectangular array case, the transmittance spectrum is also smooth, except a resonant peak at a wavelength of 0.92nm. For the Penrose lattice array, maximum transmittance occurs at two different wavelengths, at 830nm and 980 nm.

Yang et al. [46] performed a parametric experimental study by varying the array sizes from 1μm$^2$ to 20μm$^2$ on a gold film. The spectral transmission analysis indicated that the plasmonic sensing sensitivity improves as the gold-solution contact area increases. They also demonstrated that nanohole arrays with a dimension as small as 1μm$^2$ can be used to effectively detect biomolecular binding events and analyze binding kinetics.

Wang et al. [47] numerically studied the transmission spectra of metallic hole arrays with different converging-diverging channels combined with a layer of strontium titanate (STO). Simulation results showed that both the location and magnitude of the transmission peak can be tuned by temperature, converging angle of the hole, and both of the metal and STO film thickness.

Theoretical studies have been performed by several researchers to explain the EOT behavior. Medina et al. [48] performed a theoretical study using the circuit theory model to explain the extraordinary transmission theory for the case of one dimensional periodic slits. Their equivalent circuit theoretical model for this setup is in good accordance with the available numerical solution. The analytical model is able to capture the position of extraordinary peaks, Fabry-Perot resonances, and transmission dips observed in compound structures.
Martin-Moreno et al. [49] developed a three-dimensional theoretical study of the EOT through subwavelength hole arrays in optically thick metal films. The analytical data was compared with the experimental data. Their theoretical model was able to capture experimental transmission spectra of 2D penrose lattice of holes. Their analytical model shows that the enhancement of transmission is due to electromagnetic waves formed on each metal-dielectric interfaces tunneling through NHAs.

Catrysse et al. [50] used an analytical approach to study the interaction of different pathways by which extraordinary transmission through nanoscale aperture arrays arises and incorporated both propagating plasmonic and surface plasmon modes.

Marques et al. [51] performed an analytical approach using equivalent circuit theory model for the problem of EOT of electromagnetic waves through opaque screens perforated with subwavelength holes. Their analytical approach revealed that one of the key factors behind EOT is the continuous increase of excess electric energy around the holes as the frequency approaches the onset of some of the higher order modes associated with the periodicity of the screen.

Recently, Baida et al. [52] showed that one can alter the wavelength spectra of the transmission through the annular subwavelength apertures using a cascaded structure of silver metallic films. They performed a numerical study on a cascaded structure of metallic nano-layers spaced by vacuum in between the metallic layers. This type of structure resulted in having several peak transmittances at different wavelengths.

Stark et al. [53] utilized the transmitted EOT signal to present an approach toward imaging below the diffraction barrier. Their research showed that, at least 2.5 wavelengths away from the apertures, the transmitted light exhibits subdiffraction limit irradiance patterns. They
illustrated this concept by using photolithography approach on a photoresist using collimation from subwavelength apertures to obtain higher optical resolution.

The studies mentioned above focus on the parameters effecting the transmission for a range of wavelength spectra. They show that the light transmitted are affected by the metal film thickness, subwavelength hole diameter and shape, wavelength of the incident light, and hole pattern. In the following paragraphs, studies using SPR and EOT technology as a sensor are discussed.

Brolo et al. [54] used EOT signals as a biosensor technology to monitor the binding of organic and biological molecules to a metallic surface and reported the sensitivity as 400 nm per refractive index unit. Stark et al. [55] discussed the sensitivity and resolution of nanohole array sensor technology in detail for binding reactions. Using a nanohole array chip configuration of 72 hole array, with hole diameter of 150 nm and lattice constant 350 nm, they were able to detect 10μM change in NaCl solution. This measured change implies a resolution of the monitored index of refraction for the nanohole array chip is in the order of 9.4×10^{-8} Refractive Index Units (RIU). For constant concentration and pressure, the change in the index of refraction is related to the temperature change using the Lorenz-Lorentz law and the equation of state. If the medium is water at 20°C, then the expected temperature resolution equals 0.0007°C [56].

Using different nanohole array sensors on the same chip, Ji et al. [57] demonstrated that this technology can be multiplexed on a single chip by monitoring 25 binding events simultaneously. For their experiments 10 x 10 nanohole arrays (150 nm in diameter) occupying a total area of 3.3μm x 3.3μm were embedded on an optically gold film. The nanohole sensing arrays were spaced 96μm apart to prevent interference from neighboring sensing arrays. This
spacing is dependent on the CCD camera resolution (to be able to identify two adjacent binding sites) and can be less.

Lindquist et al. [58] were able to decrease the distance between nanohole arrays leading to higher packing of sensors. Nanohole arrays can cause unwanted interference since surface plasmon waves propagate to adjacent sensing areas. Lindquist et al. [58] proposed a solution to this interference using plasmonic Bragg mirrors, blocking the undesired interference. This approach can increase the sensor placement resolution on the metallic surface.

Ozdemir and Turhan-Sayan [59] discuss using SPR as temperature sensor using a Kretschmann-Raether (KR) configuration shown in Figure 1.8. KR setup consists of incident light at an angle on a prism coated with a metal film on the base. The layer above the metal film is the sensing layer. Ozdemir and Turhan-Sayan developed a model based on Fresnel reflection for the KR configuration and has related the observed reflection to the temperature dependent dielectric constants, metallic film thickness and the dielectric constants of the three media.

![Figure 1.8: Schematic of the setup used by Ozdemir and Turhan-Sayan et al. [59.](image)]


Krishnan et al. [60] showed that transmission from subwavelength holes is dependent on dielectric constant of the thin film metal, dielectric constant of the medium in contact with the metal surface and the wavelength of the incident light. Krishnan et al. [60] used a lattice of square subwavelength holes in a metallic film, with dielectric constant \( \varepsilon_2 \), deposited on a substrate \( \varepsilon_3 \) and bounded by air or liquid on the incident light surface with a dielectric constant of \( \varepsilon_1 \). This setup is illustrated in Figure 1.9.

![Figure 1.9: Schematic of the setup used by Krishnan et al. and the temperature sensor, taken from [56].](image)

In their work, Krishnan et al. changed the dielectric constant of the surface above the metallic film \( (\varepsilon_1) \) and reported the transmitted light through the subwavelength hole array as a function of wavelength for different values of the air or liquid dielectric constant, \( \varepsilon_1 \). These data clearly demonstrate the change in transmittance at the resonant wavelengths and that it also depends on small changes in the adjacent dielectric constant, \( \varepsilon_1 \). They reported that wavelength resonance \( \lambda_{\text{peak}} \) is related to the dielectric constant of the sensing layer and the metallic film layers as:

\[
\lambda_{\text{peak}} = \frac{a_0}{\gamma} \left[ \left( \frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2} \right)^{\frac{1}{2}} - \sin \theta \right] \tag{1.13}
\]
Where, \( \varepsilon_i \) is the dielectric constant of material \( i \), \( \theta \) equals the incident angle of the monochromatic light, and \( \gamma \) is an integer constant value. Their results are illustrated in Figure 1.10.

The principle behind the temperature sensor proposed by Kowalski et al. [56] is illustrated based on the work by Krishnan et al. [60]. Kowalski et al. [56] plotted the data in Figure 1.10 at a fixed wavelength of \(~\)750 nm. The change in transmission at this and other wavelengths (750nm, 1010nm, 1100nm) is shown in Figure 1.11.

![Figure 1.10](image)

**Figure 1.10:** Experimental zero order spectra of a Au film on a quartz substrate \((\varepsilon_2=2.31)\), as a function of refractive index \( \varepsilon_1 \). The film thickness is 250 nm, the hole diameter is 200 nm and the lattice constant is \( a_0=600 \text{ nm} \), taken from [60].
This concept is very important from a sensor design point of view since these variables define the response characteristics of the sensor (i.e. linearity of the sensor, dynamic range). For illustrative purposes, three different wavelengths were chosen and transmission change was plotted as a function of dielectric constant. It is important to note different trends at different wavelengths. At 750nm wavelength, transmission does not significantly depend on dielectric constant. At 1010nm, the relation between transmission and dielectric constant is linear. At 1100nm, the dependency between transmission and dielectric constant changes direction (increases and decreases after dielectric constant value of 3).

![Graph](image)

**Figure 1.11:** The relationship of the transmitted light through a nanohole array at different wavelengths as a function of the dielectric constant using the data of Krishnan et al. [60] for gold film on a quartz substrate, taken from [56].

These results demonstrate that the SPR is related to the changes to the dielectric constant, which is dependent on temperature, pressure, and composition of the adjacent layer. The effect on the index of refraction and EOT from changes in concentration has been previously...
demonstrated by Stark et al. [55] and Ji et al. [57]. Kowalski et al. [56] demonstrated the effect on index of refraction and changes in EOT from temperature changes in the EOT from the temperature changes analytically and experimentally by stabilizing concentration and light source variations.

Kowalski et al [56] has coupled the thermodynamic equations of state and the Lorentz-Lorentz law to provide a model to predict the temperature dependence of the transmittance through a nanohole array. The generalized change in the dielectric constant can be expressed as

$$\frac{d \varepsilon}{dT} \left. \right|_{P,C} dT + \frac{d \varepsilon}{dP} \left. \right|_{T,C} dP + \frac{d \varepsilon}{dC} \left. \right|_{P,T} dC$$ (1.14)

where $T$ is temperature, $P$ is pressure and $[C]$ is the concentration of the material. For the nanohole array sensor, concentration of the fluid layer is held constant and the last term in Eq. (1.14) is zero. The dielectric constant of the sensed dielectric layer, layer 1, is related to the complex index of refraction and is density dependent as shown by the Lorentz-Lorentz law.

$$\frac{\varepsilon_1 - 1}{\varepsilon_1 + 2} = \frac{n_1^2 - 1}{n_1^2 + 2} = C_0 \rho$$ (1.15)

where $C_0 = \left[ \frac{n_0^2 - 1}{n_0^2 + 2} \right] \left( \frac{1}{\rho_0} \right)$, $n$ is the index of refraction, $\rho$ is the density, $n_0$ and $\rho_0$ are the values for reference temperature and pressure conditions.

The density is related to changes in temperature and pressure of the material by the thermodynamic equation of state. The thermodynamic equation of state for solids and liquids is typically expressed in terms of specific volume, $\nu$, using the coefficient of volumetric expansion, $\beta$, and the isothermal compressibility factor, $k_T$, both tabulated properties of the material.
Based on the above equation density can be calculated as,

\[ \rho = \frac{1}{\rho} = \beta v dT - k v dP \] (1.16)

The temperature and pressure dependence can be determined by combining Eq. (1.14) and (1.17) and solving for index of refraction.

\[ \frac{\rho}{\rho_0} = \exp\{ -[\beta(T - T_0) - k(T - T_0)] \} \] (1.17)

\[ n_1^2 = \varepsilon_1 = \left[ \frac{1 + 2C_0\rho_0 \Phi}{1 - C_0\rho_0 \Phi} \right] \] (1.18)

Where, \( \Phi = \exp\{ -[\beta(T - T_0) - k(T - T_0)] \} \).

Eq. (1.18) demonstrates that as the temperature and pressure change, the optical properties of layer 1 also change.

The change in wavelength at resonance is calculated by introducing Eq. (1.18) into the wavelength formulations for wavelength at peak transmittance, Eq. (1.13),

\[ \lambda_{\text{peak}} = \frac{a_0}{\gamma} \left[ \left( \frac{\varepsilon_2 \left[ \frac{1 + 2C_0\rho_0 \Phi}{1 - C_0\rho_0 \Phi} \right]}{\left[ \frac{1 + 2C_0\rho_0 \Phi}{1 - C_0\rho_0 \Phi} \right] + \varepsilon_2} \right) - \sin \theta \right] \] (1.19)

1.4. Optical Detection Methodologies in Microfluidics

Most of the optical detection technologies require an optically transparent, non-reacting structure which provides leak free reactant delivery and allows illumination and optical monitoring in the sensing region. As discussed below, unwanted fringe patterns have been observed that have the undesirable effect of increasing the noise to signal ratio that are related to both the light source selection and microfluidic flowcell design. Microfluidic devices that are compatible with the optical detection methods have been fabricated using materials such as
polycarbonate [61] by hot compression molding and glass [62-63] by chemical etching. Polydimethylsiloxane (PDMS) technology, introduced by Whitesides et al. [64] is being widely used to fabricate complex optical surfaces and microfluidic systems [65-66] which are difficult to fabricate with other techniques.

Optical properties of PDMS were investigated by several researchers. Soni et al. [67] studied static light scattering in PDMS polymer networks. Giebel et al. [68] studied dynamic light scattering in PDMS and PMMA polymer networks. Piruska et al. [69] investigated the autofluorescence of polymers used in microfluidic device fabrication, PMMA, COC, PC and PDMS) and compared their optical properties to BoroFloat glass. Lasers at four different wavelengths were used to assess the properties, 403, 488, 532 and 633nm. The highest autofluorescence and most severe changes were observed at 403nm. For longer laser wavelengths, both the autofluorescence and its changes were reduced. Their study showed that among the four polymers, the lowest autofluorescence was exhibited by PDMS, being two times higher than BoroFloat glass. At 633nm wavelength, PDMS (3mm thick) autofluorescence intensity relative to BoroFloat was 2 (a.u.) absorbance units, and 5 a.u. at 403nm. For 3mm thick PMMA, autofluorescence intensities were 4 a.u., and 28 a.u. for 633nm and 403nm respectively. White et al. [70] reported reduction in the background signal in biosensor applications when a light absorbing material (carbon black) was introduced to the PDMS flowcell.

As discussed in [67-68], isotropic optical conditions in the PDMS flowcell can be lost in the case of a physical deformation. The induced strain inside the polymer leads to changes in the index of refraction and optical path length that causes a lensing effect within the flowcell. Deformation of PDMS microchannels was investigated by several researchers. Hardy et al. [71] used fluorescence microscopy to quantify the deformation of PDMS microchannels under
pressure driven flow. They found a linear relationship between pressure and microchannel deformation and decreasing the channel wall thickness increases the amount of deformations inside the channel. Gervais et al. [72] performed a similar analysis and used confocal microscopy to quantify channel wall deformation at different flow rates. Holden et al. [73] characterized a non-dimensional parameter to calibrate measurements taken in bulging channels. This parameter was used to convert concentrations measured in a deformed system to a rigid-wall channel measurement. The calibration parameter can predict the experimental concentration profile based on the numerical simulation within 8% accuracy.

Song et al. [74] utilized pressure induced deformation in PDMS channels as an optofluidic pressure sensor. A portion of the microchannel was designed as a flexible membrane, deforming freely in an air-gap region. The deformation of the flexible membrane results in variation in the air-gap thickness. Upon illumination by monochromatic light, the reflected light from the air-gap boundaries interferes with each other constructively or destructively, depending on the air-gap thickness. The resulting total reflection intensity can be expressed as,

$$I = I_1 + I_2 + 2\sqrt{I_1 I_2} \cos\left(\frac{4d\pi}{\lambda} + \pi\right)$$  \hspace{1cm} (1.20)

Where $I_1$ and $I_2$ are the reflection intensity from the bottom and top PDMS–air boundaries respectively and $d$ is the air-gap distance. In their setup, the air-gap chamber has a round shape, the PDMS membrane is deformed to a dome shape upon applied pressure resulting in multiple concentric interference rings similar to the Newton’s ring pattern. This interference pattern changed due to pressure variations and was captured to a CCD camera.

In combination with the flowcell material, the light source is also an important factor on the image quality. Lasers and light emitting diodes (LEDs) are widely used in SPR based sensing
platforms. Wilkop et al. [75] used a LED light source to monitor protein binding reactions using SPR imaging. In their work, they reported that LED offers an ideal point source that can eliminate interference artifacts and speckles found when using a laser source. O’Brien et al. [76] compared LED and laser light sources for a SPR array biosensor based on spectroscopic imaging. They reported that for laser light sources a rotating diffuser is very important and prevents significant speckle and interference patterns in the light that would appear on the CCD array. These patterns were not observed with a LED light source. It should be noted that, in both studies, light travels through a glass prism (not PDMS as in the case of Song et al.’s and the setup used in during this research). It is important to note that with an incoherent light source, such as LED, and a deformed channel wall, fringes can be still present as Song et al. [74] presented in their work.
2. APPROACH / METHODS

In this section, experimental setups, fabrication techniques for the components used and the analytical methodology derived for calorimetric calculations are derived. First, the experimental setup including the four port reaction chamber and PMT sensor are explained. Next, the current experimental including the T-Sensor flowcell and CCD camera are presented. The fabrication procedure for NHA and theoretical formulation for calorimetric calculations in a T-Sensor configuration are described.

2.1. Four Port Reaction Chamber

2.1.1. Experimental Setup with PMT sensor

A photograph of the overall experimental setup is shown in Figure 2.1. A 632nm Helium-Neon laser is used as the light source (Melles Griot, 05-LHP-991) (not shown in the figure, is on the left to the setup), and the light transmitted through the sub-wavelength nanoholes is measured by a photomultiplier tube (PMT) detector (Hamamatsu, H6780). Flow cell assembly consists of a glass chip with chromium bonding layer and gold film coated on top and a PDMS layer on top with the flowcell details inside. The glass chip and the PDMS sit on a thermoelectric heater. The thermoelectric heater (Melcor, SH 1.4 -15 -045) is connected to a controlling unit (Wavelength Electronics LFI-3751) to control the temperature of the flow cell unit at the thermistor location with an accuracy of ±0.002°C. Thermistors (U.S. Sensor Corp., 30-kΩ microthermistor, USP 5506, diameter of 0.450mm) are used to monitor temperature at the desired locations and to send temperature feedback for the temperature controlling unit.
Figure 2.1: Photograph of the experimental setup (Four-port) including the syringe pumps, stage the flowcell assembly and the photomultiplier tube sensor.

The temperature controlling unit is used for temperature versus EOT signal calibration experiments [56]. Figure 2.2 illustrates the schematic view of the flowcell assembly. The dielectric material (i.e. air, water, or solvent) is in contact with the gold film layer with the nanohole array sensor. All layers are tightened using a clamp type assembly. The clamp and the base are also printed on a 3-D solid object printer. Individual elements of the assembly are shown in Figure 2.3. The PDMS layer on the top acts as an insulative layer.
Figure 2.2: Schematic layout of the device (Top view).

Figure 2.3: Device assembly. From left to right: ABS plastic clamp, PDMS layer with the flowcell, glass substrate with gold film and nanohole sensors, thermoelectric heater, ABS plastic base, PMT detector.

The nanohole array sensor locations on the chip and a detailed SEM image of a nanohole array are presented in Figure 2.4. NHA chip used in this experiment had 10x10 arrays of 150nm
diameter holes with a pitch distance of 350nm. There were 25 NHA sites in total in a 5x5 configuration. The distance between each NHA site was 96μm. Figure 2.5 shows the detailed view of the nanohole array chip and the PDMS flowcell. The nanohole array sensors are in the center of the trapezoidal reaction chamber. The volume of the reaction chamber in this configuration is 8.8μL. In this case, the volume value is determined by the resolution of the 3-D solid object printer (300μm for our case).

Figure 2.5 also shows the inlets and the outlets of the flowcell. Ports labeled as 1 and 2 are used as inlet ports, introducing the reactants. Ports labeled 3 and 4 are used as outlet ports, used for disposal and flushing of the reactants. Ports 1 and 2 are connected to 10 mm diameter syringes. Each syringe is connected to a syringe pump (Harvard Apparatus 11P and Harvard Apparatus 4400-001) to control the injection volume and speed. Data is collected using LabView 8.0 software and MATLAB is used for post processing.

![Figure 2.4: Details of nanohole array chip and SEM image of a nanohole array.](image)
2.1.2. Flowcell Fabrication

The microfluidic delivery system and the flowcell is created using micro molding process as outlined by McDonald et al. [77]. The processes followed in this study are summarized in Figure 2.6. The mold design, with the feature details of the flowcell unit are designed in Solidworks CAD program. Next, these designs are printed by a 3-D prototype SLA plastic printer (3D Systems Viper Si 2, SLA Accura 40 Resin material). Next, molds are cleaned with isopropanol, dried with Nitrogen gun and filled with the PDMS mixture (10:1 ratio of base and curing agent). After the pouring process, PDMS in the molds are degassed in a vacuum chamber and baked at 65°C for three hours. This procedure allows great flexibility in the mold and hence the flowcell design and reduces the design and turnaround time significantly. In addition, the molds can be used again. The disadvantage of this process is the resolution. The channel height is 300µm in all of the designs. The minimum feature size is limited by the 3-D printer resolution (300µm for our case).
2.2. T-Sensor Type Design

In this sub-section, the experimental setup for the T-Sensor type microfluidic flowcell is described. The T-Sensor type design was introduced due to the uncertainty in the mixing mechanism and inefficient mixing mechanism of the four port flowcell. The T-Sensor type design has an analytically predictable mixing mechanism based on the laminar diffusion interface. This property made it possible to identify the heat induced signal change during a reaction.

2.2.1. Theoretical Development - Calorimetric (Energy) Equations in T-Sensor Configuration

This section starts with a general form of the energy equation for a uniform flow in z-direction. The coordinate system is shown in Figure 2.7. The z-direction is the flow direction, y-direction is normal to the chip surface and x-direction represents the channel width.
Starting with the three dimensional, steady state differential energy balance:

\[
\sum_j \left( \rho c V_j \right) \frac{\partial T}{\partial z} = k \left( \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} \right) + q_{\text{gen}}(x, y, z)
\]  

(2.1)

Where the heat generation term, \( q_{\text{gen}} \), represents the energy added or removed from the control volume due to the enthalpy of reaction for the species. Integrating in y-direction, from \( y=0 \mu m \) to \( y=d \), and using the definition for the average temperature over the y-direction, \( \bar{T} = \frac{1}{d} \int T dy \), reduces the energy balance to the following form:
\[
\int_{0}^{y=d} \sum_{j} (\rho c V_j) \frac{\partial T}{\partial z} \, dy \\
= k d \left( \frac{\partial^2 \bar{T}}{\partial x^2} + \frac{\partial^2 \bar{T}}{\partial z^2} \right) + k \int_{y=0}^{y=d} \frac{\partial^2 T}{\partial y^2} \, dy + \dot{q}_{\text{gen}}(x, y, z) \, d
\]

(2.2)

The y-directed conduction integral on the right hand side can be evaluated and the boundary heat flux terms introduced into the energy balance.

\[
k \int_{y=0}^{y=d} \frac{\partial^2 T}{\partial y^2} \, dy = k \left. \frac{\partial T}{\partial y} \right|_{y=d} - k \left. \frac{\partial T}{\partial y} \right|_{y=0} = -q''_{y=d} + q''_{y=0}
\]

(2.3)

Numerical results show that, \( q''_{y=0} \gg q''_{y=d} \), and the heat loss on the PDMS side, \( y=d \), is neglected. Next, integrating in x-direction, from \( x=0 \mu m \) to \( x=w \), introduces the x-directed boundary heat flux terms into the energy balance.

\[
\int_{0}^{x=w} \int_{0}^{y=d} \sum_{j} (\rho c V_j) \frac{\partial T}{\partial z} \, dy \, dx \\
= k w d \frac{\partial^2 \bar{T}}{\partial z^2} + k d \int_{x=0}^{x=w} \frac{\partial^2 \bar{T}}{\partial x^2} \, dx + \int_{x=0}^{x=w} q''_{y=0}(x, z) \, dx \\
+ d \int_{x=0}^{x=w} \dot{q}_{\text{gen}}(x, z) \, dx
\]

(2.4)

In the equation above, \( \bar{T} \) represents average temperature over x-direction (channel width) and \( y \) and \( \bar{T} \) represents averaged temperature over y-direction (normal to the flow surface). As explained in the previous sections, the boundary heat transfer in x-direction is negligible,

\[
k \int_{x=0}^{x=w} \frac{\partial^2 \bar{T}}{\partial x^2} \, dx = k \left. \frac{\partial \bar{T}}{\partial x} \right|_{x=w} - k \left. \frac{\partial \bar{T}}{\partial x} \right|_{x=0} = 0
\]

(2.5)
and the integrated energy balance is:

\[
\int_0^{x=w} \int_0^{y=d} \sum_j (\rho c V_z j) \frac{\partial T}{\partial z} \, dy \, dx = \int_0^{x=w} q_{y=0}''(x, z) \, dx + d \int_{x=0}^{x=w} q_{gen}(x, z) \, dx
\]

\[\text{(2.6)}\]

The term, \(q_{y=0}''\), represents the heat flux coming from the chip surface and can be stated in terms of the heat loss coefficient, \(H\), using the heat sink temperature, \(T_{set}\), on the back chip side of the device at \(y=-0.670\text{mm}\). The heat flux term inside the integral can be defined as:

\[q_{y=0}''(x, z) = H(x, z) [T_{set} - \bar{T}(x, z)]\]

\[\text{(2.7)}\]

The total heat source is evaluated by integrating along the flow direction (in \(-z\)), between the locations \(z_1\) and \(z_2\):

\[
d \int_{z_1}^{z_2} \int_{x=0}^{x=w} \dot{q}_{gen}(x, z) \, dx \, dz = \int_{y=0}^{y=d} \left( \int_{x=0}^{x=w} \sum_j (\rho c V_z j) (T(z_2) - T(z_1)) \right) \, dy
\]

\[\text{-(2.8)}\]

The total amount of energy absorbed/released is related to the enthalpy of reaction of the product, \(\Delta H_{AB}\):

\[\Delta H_{AB} = \frac{d \int_{z_1}^{z_2} \int_{x=0}^{x=w} \dot{q}_{gen}(x, z) \, dx \, dz}{(\dot{m}_{\text{outlet}} - \dot{m}_{\text{inlet}}) \, M_{AB}}\]

\[\text{(2.9)}\]
This section summarizes the analytical approach using the general form of energy equation. Emphasis has been kept on the heat source term and necessary simplifications have been done based on the previous numerical results. Eq. (2.9) shows that enthalpy of reaction \( \Delta H = \frac{q}{n_{AB}} \) can be extracted based on temperature measurements of the flow field for this experimental configuration.

### 2.2.2. Experimental Setup with CCD Camera

The experimental setups are shown in Figure 2.8 (a) and (b) for two different light sources. For the experiments with laser light source, a He-Ne laser (Melles-Griot, 10mW) was used as a constant wavelength (632nm) light source and the beam traversed a water-filled flowcell to illuminate the NHA sensors. An optical mirror was used to illuminate the chip at different incident angles. For the experiments with LED, a red collimated LED light source (Thorlabs, M625L2-C1) with maximum power intensity at 625nm wavelength was used. Both light sources were sent through a polarizer/beam splitter. A PMT (Hamamatsu, H6780) sensor was attached to beam splitter to monitor the intensity drift of the light source. A condenser lens was placed in between the polarizer and flowcell to convert the Gaussian profile of the LED light intensity to a uniform intensity profile.

The EOT signal through the NHAs with 150nm diameter holes was measured using a CCD camera (Retiga 4000-R, QImaging) with 10x microscope objective lens (Nikon). The 12-bit CCD camera digitizes intensity measurements to 4096 gray levels. The optical setup and the CCD camera allowed us to measure transmission through 38 NHA sites simultaneously.

LabVIEW software was used to measure the intensity values at each NHA site and record to an excel sheet. The center pixel of each NHA site and the size of averaging region were given
to the LabVIEW as an input. Using LabVIEW, the gray level in the vicinity of the center of the NHA site was measured and averaged after each reading. This approach was used to increase reading speed and also save on the memory space. This was helpful in terms of saving and performing calculations only in the regions of interest (NHA sites) and not taking or recording entire image of the viewfield.

![Schematic of the optical setup](image)

**Figure 2.8:** Schematic of the optical setup of (a) He-Ne laser light source, (b) LED light source. Red light indicates the optical path.

The components of the flowcell assembly are presented in Figure 2.9. The assembly is designed to enable introduction of small volumes of reactants to a 25 nL chamber in contact with a sensor chip. Starting from the left, the assembly consists of the top part of the clamp, glass
cover, silicone flowcell, NHA chip, copper heat sink (35mmx35mm), thermoelectric heater and the clamp base. All components were clamped together using screws. The assembly was mounted on a rotational XYZ stage.

A photo of the copper heat sink, NHA chip and PDMS flowcell is shown in Figure 2.10. The temperature of the copper heat sink was regulated using a thermoelectric heater with a temperature controller. The sensing chip is in direct contact with the copper heat sink. A thermistor (wire shown under the black tape, 0.450mm in diameter) was used to maintain a constant temperature of the upper surface of the copper heat sink during the experiments and send feedback to the temperature controller. Care was taken during the mounting procedure and the pressure was applied by the outer mounting plate (Fig. 3) to ensure good, consistent thermal contact between the thermistor and the heat sink. Long termed observations of this temperature and the heater power dissipation supports that consistent thermal contact was maintained with this mounting procedure. The temperature controller has a reported accuracy of +/-0.002°C and was used to perform step changes in temperature of the system.
The flowcell was fabricated by casting poly-dimethylsiloxane (PDMS) elastomer (Sylgaard 184;10:1 prepolymer:curing agent) over a micro-fabricated template which was fabricated at Draper Laboratory. It was in direct contact with the gold chip surface to ensure sealing. There are two inlet ports and one outlet. The width and height of the microchannel reaction chamber is 500µm and 10µm respectively. To prevent sagging on the upper wall of the PDMS flowcell due to high width/height ratio, the PDMS flowcell included cast posts inside the channel. The 15 um diameter posts were spaced on a regular grid, 180 um apart throughout the reaction chamber. Their locations did not necessarily correspond to the NHA site locations.

![Image of the PDMS flowcell, NHA chip with gold surface, copper heat sink.](image)

Figure 2.10: Image of the PDMS flowcell, NHA chip with gold surface, copper heat sink.

Two different T-sensor type flowcell designs were tested. These designs are shown in Figure 2.11. The first design had PDMS in the light path and has a channel height of 10µm. The
second design had the same flow channel geometry and PDMS was used as a 100µm thick gasket between the glass cover and sensing chip. Second design had no PDMS in the optical path. The main motivation for switching from the design in Figure 2.11 (a) to the design in Figure 2.11 (b) was to eliminate the PDMS from the illumination path and prevent interference due to sagging in the upper PDMS channel wall. This behavior is described in detail in the Results section.

![Figure 2.11: Cross sectional view of the different flowcell designs, (a) 2mm thick PDMS with 10µm thick flow channel, (b) 100µm PDMS gasket.](image)

### 2.3 Nanohole Array Sensor Fabrication

The focused Ion Beam (FIB) method is used to for the nanohole array chip fabrication. This method is outlined in previous studies [54, 57]. The metal film deposition on the glass wafer and nanohole perforation were performed in Harvard Center for Nanoscale Systems (CNS). NVision 40 CrossBeam Focused Ion Beam (FIB) system is used for the fabrication. The
procedure followed is as follows: A glass slide is cut to desired dimensions (8.2mm x 31.8mm) using Automatic Dicing Saw (DISCO ADA-321). Next, metal deposition is done to the glass slides using E-Beam Evaporator. The glass slides are coated with first 25nm Chromium (Cr) and then 105nm gold (Au). Next, nanohole milling is performed on the FIB. Nanohole array specifications are taken from the work by Ji et al. [57]. The diameter for each hole is 150nm and the pitch distance between each hole is 350nm. Each array consists of 10 x 10 nanohole arrays and the distance between the arrays are 96µm. For this case, 2x2 array of 10x10 nanohole array configuration is fabricated. One sensor, 10x10 array is shown in Figure 2.12. The hole pattern is prepared in AutoCad software and saved in .bmp format.

Figure 2.12: FIB image of a 10 x 10 nanohole array.
Necessary beam current and the dosage time are determined using the following procedure. 1pA current is applied to the sample at different dosage times ranging from 10 to 20 seconds. Next, a rectangular groove is opened next to these holes to view them in cross section and the depth is measured. Figure 2.13 presents the results. In this process, avoiding over dosage of Galium Ions is important since the Galium Ions will be deposited to the exposed glass surface and hence affect transmittivity of the glass in case of an over exposure. The results show that 15 seconds is necessary to mill through the layers of Gold and Chromium with an ion current of 1pA.

Figure 2.13: Ion beam dosage time determination.
3. RESULTS AND DISCUSSION

3.1. EOT Experiments without a Flowcell (Bare Chip)

This section discusses the concept of EOT based on our experimental measurements. These measurements were performed with a bare chip (no flowcell) and 632nm wavelength laser as the light source. The diameter of the holes was 150nm and the array size was 10x10 with 350 pitch distance.

The motivation behind this experiment was to verify EOT behavior and compare EOT intensity measured in our experimental setup to the Bethe’s aperture transmission theory. The experimental procedure was as follows. The exposure was kept at constant value of 450ms and an optical density filter (ODF) of 1.8 was used to lower the incident laser power (to avoid saturating the CCD camera). The direct laser intensity was measured first (no chip in front, just the camera lens) and next chip was placed in front of the camera and all the other parameters were kept constant. At the same exposure rate, the EOT measurements were taken from the NHA sites. Figure 3.1 and Figure 3.2 show the intensity profiles measured using CCD camera with and without the chip. The CCD camera digitizes intensity level to a number between 0-4095 (CCD units) based on the gray levels. The incident intensity without the chip was measured as 3768 CCD units and the maximum averaged transmission from the NHA site was measured as 60 CCD units.

Transmission efficiency for a single aperture, predicted by Bethe [39] is:

\[
\eta_{\text{Bethe}} = \left( \frac{\Gamma}{\lambda} \right)^4 = \left( \frac{75}{632} \right)^4 = 1.983 \cdot 10^{-4}
\]  

51
where $r$ is the radius of a nanohole and $\lambda$ is the wavelength of the incident laser. Using this value and the measured incident laser light, Bethe’s predicted transmission is:

$$\text{Predicted transmission} = 1.983 \cdot 10^{-4} \cdot 3768 = 0.747 \text{ CCD units} \quad (3.2)$$

Using the same laser intensity, the measured value for the EOT transmission was 60 CCD units, which was larger than the above calculated value as expected in EOT behavior.

Figure 3.1: (a) CCD image of the NHAs in the center (gray dots), (b) CCD image of the laser intensity when there is no chip in the laser path.
Figure 3.2: (a) Intensity profiles plotted across the NHAs. The spikes correspond to individual NHA sites. (b) Laser intensity profile across the same pixels location when there is no chip.

EOT transmission was calculated by dividing averaged EOT transmission at the NHA site to the measured laser intensity value at the corresponding location. The transmission calculation is shown in Eq. (3.3).

$$\eta_{EOT} = \frac{60}{3768} = 0.016$$  \hspace{1cm} (3.3)

Since the transmission was over a NHA site (not through a single hole but an NHA array), this value can be normalized by the fraction of nanohole area compared to the NHA site.
to obtain the absolute transmission efficiency defined by the division of the fraction of light transmitted by the fraction of surface area occupied by the holes [40]. This normalization was done in order to compare the results with the aperture theory. Our array parameters were, pitch, $p$, 350nm, hole diameter, $d$, 150nm, array size $N \times N$, 10x10. The parameters are illustrated in Figure 3.3.

$$\frac{\text{Area of holes}}{\text{Area of NHA site}} = \frac{(N \times N) \cdot \left(\frac{\pi \cdot d^2}{4}\right)}{[(N - 1) \cdot p + d]^2} = 0.162 \quad (3.4)$$

EOT transmission can be compared to the Bethe’s prediction using the values calculated:

$$\frac{\eta_{\text{EOT}} \cdot A_{\text{holes}}}{\eta_{\text{Bethe}} \cdot A_{\text{NHA}}} = \frac{0.016 \hspace{1em} \text{CCD units}}{1.983 \times 10^{-4} \hspace{1em} \text{CCD units}} = 499.24 \quad (3.5)$$

Eq. (3.5) shows that the EOT transmission exceeds the predicted area based value, as expected by the aperture theory.

**Figure 3.3:** A sketch of a single NHA site. Transmission occurs not only through the holes but through the entire rectangular region.
3.2. Four Port Reaction Chamber Results

3.2.1. EOT versus Temperature Calibration

To study and understand the properties of the nanohole array chip, temperature versus EOT signal plots have been created using the setup in Figure 2.2 for air and water. For this experiment, temperature in the flow cell with air inside has been increased from 28°C to 42°C in steps of 2°C and decreased in the same manner and the EOT signal has been recorded. The same procedure has been repeated after injecting water into the flow unit. This particular setup involves no fluid motion once the injection is completed. For these experiments concentration does not change, hence the dielectric constant change is only due to temperature changes. This procedure is described in detail in the work by Kowalski et al. [56].

The steady state results for the experiment are shown in Figure 3.4. Figure 3.4 (a) shows the controlled temperature, and the step changes. Figure 3.4 (b) presents the steady EOT response. These results were obtained by taking the steady section of the EOT signal after the temperature change. A steady state condition at each temperature was achieved by keeping the system at the set temperature for 20 minutes. The last 300 EOT data points during a set temperature were considered as the steady state signal. These data points are presented in Figure 3.4 (b). Figure 3.4 (c) represents the temperature calibration curve, the cross plot of the steady EOT signal and temperature using steady state values.
3.2.2. Numerical Analysis

3.2.2.1. Numerical Setup

Numerical simulation of the four port reaction chamber was performed to analyze the thermal characteristics of the system. This includes the transient thermal response of an
imaginary EOT plane on the sensor region with a commercial thermistor. The same simulation is also used to analyze the effect of temperature gradients in the system and to design an isothermal copper heat sink to ensure isothermal reactant delivery. Fluent commercial CFD software was used to solve the equations and the Gambit software was used to build and mesh the geometry.

3.2.2.2. Transient Numerical Analysis

The numerical simulation for the system is shown in Figure 2.2. The numerical simulation was based on a 20,988 node system in which the transient temperature response of the system was determined for switch on heater value of 210 W. The 3D geometry and the grid setup are shown in Figure 3.5. The boundary and heat input conditions correspond to the laboratory conditions.

![Figure 3.5: Grid and the geometry for the transient analysis.](image)

The spatially averaged temperature results for thermistor, fluid cell water and EOT sensing region over the first five seconds of the response are shown in Figure 3.6 and clearly
illustrate that at the start of the thermal response there are significant differences in the spatially averaged temperatures of these volumes. These differences are due to the transient heating and energy storage effects which are part of the response time of the EOT and thermistor sensors.

Figure 3.6: Numerical simulation results describing the spatially averaged temperature response of the EOT sensor volume, fluid cell volume and the thermistor volume for the first 200 seconds. The boundary conditions and heat input correspond to the experimental conditions.

The complete transient response for the EOT sensor volume (solid line) and thermistor (open square symbols) is shown in Figure 3.7. These results demonstrate that there is a negligible steady state temperature difference between the EOT sensor volume and the thermistor for the expected heat flow rates in the experiment. The plotted results also illustrate that the observed transient response is governed by the large thermal mass of the heaters, and glass substrate and fluid cell material and not by either the EOT sensor volume or thermistor thermal mass. The
long response time of 3700 seconds is reflective of the system response and not of the sensor response. The EOT sensor volume is responding faster than the thermistor as expected from the preceding discussion. The thermistor has a significant lag at the start of the process. The temperatures for the regions do approach each other at long times. The purpose of this investigation was to provide experimental verification that the nanohole array sensor as constructed for this experiment can be used as a small temperature sensor that is inherently fast.

![Graph](image)

**Figure 3.7:** The numerical simulation results describing the spatially averaged temperature response of the EOT sensor volume and the thermistor volume. The boundary conditions and heat input correspond to the experimental conditions [56].

### 3.2.2.3. Isothermal Reactant Delivery Numerical Analysis

The numerical analysis was performed in the presence of a constant temperature heating source to determine spatial temperature variation on the chip, which can be as much as ~3.5 K
along the chip. Further analysis was used to design a copper heat sink between the heater and the sensing chip to eliminate the temperature gradient.

Figure 3.8: Temperature in the flowcell in the x direction.

As seen in the Figure 3.8, starting from the inlet, x=13.70mm, there is a ≈3.5K temperature difference in x direction. Figure 3.8 shows the temperature on the flowcell / chip interface in the x direction. The temperature of the heater surface is also plotted. This large temperature gradient across the sensing chip is not desirable for a reactant delivery system since it adds an unknown, uncontrolled internal energy change to the observed thermodynamic changes in the flow cell.

A copper plate with dimensions of 60mm x 35 mm x 5mm is used as the heat sink and is placed between the heater and the chip in the numerical simulation of the system with the heat sink. The system is solved for steady state conditions and at a constant temperature defined at the
heater surface, similar to the experimental protocol. Figure 3.9 shows the steady state temperature plot of the flowcell / chip interface along the x axis. The upper horizontal data corresponds to the temperature distribution of the heater surface. Comparing Figure 3.8 and Figure 3.9, it can be clearly seen that the steep gradient in the entrance region of the flowcell is eliminated for the copper heat sink case.

Figure 3.9: Temperature in the flowcell along the x direction with a copper heat sink between the heater and the sensing chip.

Figure 3.9 shows that variation across the sensing chip surface is ≈ 0.03K, which was ≈ 3.5K for the first case. Based on this result, it was concluded that, with the copper plate heat sink, the sensing chip surface is close to the isothermal surface conditions. This condition is highly desirable, since it ensures that the reactants will be delivered at the same temperature, eliminating the external heat addition effects during the injection until the fluid reaches flowcell.
In addition, in terms of calorimetry, delivering the reactants at the same temperature also eliminates the additional heat diffusion in the flowcell while two reactants at two different temperatures will come to an equilibrium temperature. In other words, isothermal delivery of reactants also ensures that the heat measured in the flowcell is due to the reaction mechanism.

3.2.3. Calorimeter type results

The experimental setup is shown in Figure 3.10. The thermoelectric heater surface is kept at a constant temperature (30°C in this study) using the temperature controller. The temperature feedback is given by a thermistor placed on the heater surface. A second thermistor is inserted through the first inlet port and measures the incoming liquid temperature.

![Figure 3.10: Experimental setup and thermistor locations.](image)
To check the repeatability and the monitoring capability of the EOT signal due to temperature change, the following experimental procedure was followed. The system is filled with Deionized water, and brought to steady state at the set temperature. Once the system reached steady state temperature, room temperature water is injected with volume of 16µL and at a speed of 0.1mL/min. The transient response of EOT signal, temperature information from the two thermistors, current and the voltage (hence power) response of the thermoelectric heater is measured in real time and recorded through LabView software. This room temperature push is repeated several times after system has reached steady state conditions. Next, for the EOT vs. temperature calibration purposes, the thermoelectric temperature heater is set to different temperatures (29°C to 31°C, in steps of 0.2°C) and the EOT signal is recorded at the steady state level. This procedure is described in detail by Kowalski et al. [56].

In order to estimate the temperature in the flowcell region for calibration purposes, a numerical model of the system is used [78]. For validation of the numerical model temperature values in the reservoir region are compared from experimental and numerical systems for different heater set temperature values. These values are reported in Table 3.1. The difference is within 0.1K.

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Table 3.1: Comparison of the experimental and numerical data.
Using the numerical simulation, the flowcell temperature is estimated for the set temperature used in the experiment. This temperature information is used with the EOT signal information to convert EOT signal to temperature values. The calibration part of the experiment is presented in Figure 3.11. The blue line represents the thermistor measurement in the reservoir region (inlet port). The red line represents the controlled temperature at the heaters surface. The black line represents the temperature of the flowcell estimated using the EOT signal.

![Graph](image)

**Figure 3.11:** Calibration experiment, temperature in different regions plotted with respect to time. Red line represents controlled temperature, black line temperature in the flowcell estimated by the EOT, blue line represents the reservoir temperature.

Results from the injection experiments are presented in Figure 3.12. The temperature in the flow cell is slightly lower than the heater surface and higher than the reservoir temperature. This temperature difference is due to the geometry of the heater and the system. Figure 3.12 suggests that EOT signal is able to follow the transient changes in temperature in the flow cell region and follows the expected trend in a repeatable manner. Each pulse represents an injection
with the same volume and same rate of DI water at the room temperature. The lower plot shows the heater power input (in Watts) due to the injections.

![Graph showing temperature and power over time]

**Figure 3.12:** Water injection experiment results. Red Line represents Heater Temperature, Black Line represents Flowcell Temperature measured by EOT signal converted with Figure 3.11 information, Blue Line represents reservoir temperature measured by thermistor (Top Figure).

Figure 3.13 shows the individual EOT responses from each injection. The temperature conversion from EOT was performed by extrapolating the numerical values listed in Table 3.1. These signals are plotted on top of each other by normalizing the signal and the time to present
the difference between similar injections. The results show that the signal is repeatable; and maximum and minimum deviations from the mean are +0.15°C and -0.20°C respectively.

Figure 3.13: Normalized EOT signals plotted on top of each other with respect to normalized time.

Figure 3.14 shows the transient response of EOT signal compared to the 450µm diameter thermistor (U.S. Sensor Corp., 30-kΩ microthermistor, USP 5506). The temperature measured from thermistor and EOT signal are normalized to make the comparison. The shorter width in the EOT signal’s pulse suggests the faster transient behavior compared to the thermistor used in the
experiment. The EOT signal goes back to the steady state after 23 seconds, thermistor signal goes back to steady state after 150 seconds.

![Figure 3.14: Transient response of the thermistor and the EOT signal for the 3\textsuperscript{rd} injection.](image)

For the next experiment, saturated salt solution is introduced from the inlet channel for a salt dilution experiment. Heat of dilution of NaCl solution has been used for calibrating industrial isothermal titration calorimeter devices by Tellinghuisen [79]. For this experiment, the flowcell is filled with DI water initially and the inlet channel is filled with saturated salt solution and the system is brought to 30°C. Each injection consists of 4µL of saturated salt solution
introduced at a rate of 0.1mL/min. After each injection transient signals are recorded. Once the system reaches steady state, second injection is done. The thermistor locations are illustrated in Figure 3.10.

Figure 3.15: EOT response of saturated salt solution injections. Each pulse in the plot represents an injection of 4µL saturated salt solution at a rate of 0.1mL/min.

Figure 3.15 shows the EOT signal response for the complete experiment. Each pulse represents a push of saturated salt solution. It should be noted that the EOT signal in this experiment is dependent on both concentration and temperature. Red lines represent the averaged steady signal values for concentration calculations. The last push is the pure water injection which was used to purge the system. It can be observed that EOT level goes back to the initial level as expected since the system is filled with water similar to the initial state. Detailed response characteristics of the heater thermistor and power are presented in Figure 3.16, the upper plot shows the temperature on the heater surface. The injections result a temperature
change smaller than 0.015°C on the heater surface. The plot in the middle presents the heater response in power (Watt) units. The change in the temperature from the reaction results in a change in the dissipated heated power. The bottom plot presents the EOT signal, representing the temperature and concentration change in the flowcell due to the salt injection. The reaction due to the salt injection ends after 20 seconds for the first and 15 seconds for the second injection. The signal goes to a steady level after each injection. The steady level increases after each injection and is due to the increasing concentration in the flowcell.

Figure 3.16: Results from salt injections. Response characteristics of the thermistor and heater along with the EOT signal.
A titration type of result can be obtained by monitoring the EOT level as the concentration changes after the thermal effects have dissipated (as shown in red lines in Figure 3.15). For this purpose, the salt concentration in the flow cell at each injection is calculated using the following mass balance,

\[
m_{NaCl}^{t+1} = m_{NaCl}^t + m_{NaCl}^{injected} - \frac{m_{NaCl}^t}{V_{flowcell}} V_{injection}
\]

(3.6)

Where \(t\) represents the time, \(m\) represents the mass of the NaCl present in the flowcell and \(V\) represents the volume of the region. This approach is similar to the concentration calculations in the reaction chamber for the ITC calorimetry [79].

![Figure 3.17: Comparison of steady state concentration values with theoretically calculated concentration values.](image)
The theoretical curve obtained from the Eq. (3.6) and the averaged steady EOT signals after each injection signals are normalized and plotted in Figure 3.17. These results suggest that a titration type of experiment can be performed using this device. The errors are due to the compliance issues in the delivery system and mixing problems in the flowcell.

The preliminary results show that, continuous temperature changes can be monitored using the changes in the EOT signal. A transient response comparison is done experimentally with an industrial thermistor device and it has been shown that EOT has faster response capability. Water injection tests shows that EOT signal is repeatable if similar conditions are present. The flowcell volume used in this study could be further reduced by photolithography methods since the footprint of a sensor is only 3.3µm x 3.3µm. In our study, the volume of the flowcell is limited by the printer resolution. Salt dilution tests show that, EOT signal can continuously monitor the temperature and concentration changes and hence can be utilized as a calorimeter device for chemical reactions. However, since during a reaction concentration is not constant, the EOT signal should be post-processed carefully since it is affected by temperature change and concentration change. Hence, concentration change during a reaction and its effect on the signal should be also taken into account while interpreting transient results.

3.3. T-Sensor Results

3.3.1. Numerical Analysis

Numerical simulation of the laminar diffusion interface in the T-Sensor has been performed to analyze the flow and thermal characteristics of the system. A three dimensional computational analysis of the reacting flow field, species diffusion and heat transfer processes in microchannel reactors composed of coflow configuration has been performed. The microchannel
coflow geometry used in this numerical analysis is shown in Figure 3.18. The flow configuration is two parallel streams of reactants in aqueous state (dilute NaOH and HCl in this study). The Fluent commercial CFD package is used to solve for the Navier-Stokes, mass transport and the energy equations. The flow field solution is validated by comparing the velocity profile with the analytical solution. A methodology to extract enthalpy of reaction $\Delta H$, is presented by calculating the energy balance in several control volumes in the microchannel. The numerical results are used to design a nanohole array temperature sensor which is discussed in the numerical results section.

3.3.1.1. Model Geometry and Computational Domain

We constructed a numerical model corresponding to features that could be fabricated using practical techniques for a test device. A minimized channel height conserves reactant volumes. The channel width was chosen to be large enough that sidewall thermal boundary effects would have a negligible effect on the reaction and diffusion kinetics at the channel center. The channel length was restricted by the computational resources and chosen to be sufficiently long that equilibrium is established at the outlet. No further effort was made to optimize these dimensions. A representative base-acid reaction will be used in this analysis because it is typically used in calorimeter standardization. Boundary conditions were selected to simulate commonly used materials, compatible with sensor technologies and optical viewing.

The channel thickness in the $y$-direction, $d$, is 10 $\mu$m and the channel width in the $x$-direction, $w$, is 500 $\mu$m. The length in the flow direction, $L$, is 5000 $\mu$m. The chamber volume is 25nL. The total number of nodes used for this domain is 500,000. Gambit software is used for meshing. The simulation was solved in three-dimensional space, and coupled form of fluid flow, mass transport, heat transfer and the chemical reaction models were solved. Simulations were
performed on a PC (Intel Dual Core 1.80GHz CPU, 3GB RAM) and each run took approximately 48 hours to complete. For the specific reaction modeled in this study, the degree of freedom of the system was eight. To study the thermal boundary layer development, a smaller portion of the domain size was used. The fluid in the flow channel is surrounded by a glass chip on the bottom, 670 µm thick and a polydimethylsiloxane (PDMS) surface on the remaining other sides, 2 mm thick. The coordinate system used, flow direction, and the details of the layers are illustrated in Figure 3.18. This figure was repeated as a reminder of the coordinate system.

![Flow geometry of the CFD model.](image)

Figure 3.18: Flow geometry of the CFD model.
We modeled a two-species reaction of the form

\[ n_1A + n_2B \rightarrow n_5AB + n_3A + n_4B \] (3.7)

Aqueous solutions of compounds A and B each flow independently, at equal volumetric flow rate, and merge at the xy plane at z=0, the inlet plane.

In our 3-D model, no assumptions or simplifications were done on the mass diffusion equations. Conservation of mass and energy was solved in 3-D space and the heat and mass diffusion was considered in all dimensions. Hence, dispersion in the flow direction was also considered (unlike the work by Kamholz et al. [15]). The velocity profiles in the entry region reported in Figure 3.19 are consistent with the velocity profiles presented by Beard [21].

### 3.3.1.2. Governing Equations and Boundary Conditions

The continuity equation, Navier-Stokes equations, energy equations, mass transport equations and reaction rate equation are solved for the steady state case in 3-D space using the commercial CFD software Fluent (ANSYS, Canonsburg PA). Fluent uses the finite volume approach by Patankar [80]. A second order upwind scheme is applied to discretize the spatial derivatives. The continuity and momentum equations solved are:

\[ \nabla \cdot (\rho \vec{V}) = 0 \] (3.8)

\[ \nabla \cdot (\rho \vec{V} \vec{V}) = -\nabla p + \rho \vec{g} + \nabla \cdot (\vec{f}) \] (3.9)

Where \( p \) is static pressure, \( \vec{f} \) is the stress tensor, and \( \rho \vec{g} \) is gravitational body force and \( \vec{V} \) is the velocity vector.
The stress tensor $\bar{\sigma}$ is given by,

$$\bar{\sigma} = \mu \left[ (\nabla \nabla + \nabla \nabla^T) - \frac{2}{3} \nabla \nabla \right]$$  \hspace{1cm} (3.10)

Where $\mu$ is the molecular viscosity, $I$ is the unit tensor.

The energy equation is:

$$\nabla \cdot (\bar{V}(\rho E + p)) = \nabla \cdot \left[ k \nabla T - \sum_j h_j \bar{J}_j \right] + S_h$$  \hspace{1cm} (3.11)

Where $\bar{J}_j$ is the diffusion flux of species $j$. The first two terms on the right hand side of the Eq. (3.11) represent the energy transfer due to conduction and species diffusion, respectively. $S_h$ includes the energy generation from chemical reactions.

$E$ is defined as the sum of the enthalpy minus the flow work and the kinetic energy. The flow work term is included in the left side of Eq. (3.11):

$$E = e - \frac{p}{\rho} + \frac{V^2}{2}$$  \hspace{1cm} (3.12)

And for incompressible flows, $h$ is defined as:

$$e = \sum_j Y_j e_j + \frac{p}{\rho}$$  \hspace{1cm} (3.13)

Where $Y_j$ is the mass fraction of species $j$ and

$$e_j = \int_{T_{ref}}^{T} c_{p,j} dT$$  \hspace{1cm} (3.14)

with $c_p$ the heat capacity, and $T_{ref} = 298.15 \text{ K}$.
For the species transport model the governing equations are, for each species $i$,

$$\nabla \cdot (\rho \vec{V} Y_i) = -\nabla \cdot \vec{J}_i + R_i \tag{3.15}$$

For a laminar flow, mass diffusion is governed by the equation,

$$\vec{J}_i = -\rho D_{i,m} \nabla Y_i \tag{3.16}$$

Where $D_{i,m}$ is the diffusion coefficient for species $i$ in the mixture. $R_i$ is the net rate of production of species $i$ by chemical reaction and is computed using:

$$R_i = M_{w,i} \hat{R}_i \tag{3.17}$$

Where $M_{w,i}$ is the molecular weight of species $i$ and $\hat{R}_i$ is the Arrhenius molar rate of creation/destruction of species $i$. $\hat{R}_i$ is calculated using:

$$\hat{R}_i = (n_i'' - n_i') \left( k_f \prod_{j=1}^{N} [C_j]^{(\eta_j' + \eta_j'')} \right) \tag{3.18}$$

Where, $C_j$ is the molar concentration of species $j$, $\eta_j'$ is the rate exponent for reactant species $j$, $\eta_j''$ is the rate exponent for product species $j$, and $n_i'$ and $n_i''$ are stoichiometric coefficient for reactant and products respectively.

The forward reaction rate constant, $k_f$, is calculated using Arrhenius expression,

$$k_f = A_{\text{arrhenius}} e^{\frac{E_a}{R_{\text{gas}} T}} \tag{3.19}$$

Where, $A_{\text{arrhenius}}$ is a pre-exponential factor, $E_a$ is the activation energy for the reaction and $R_{\text{gas}}$ is the universal gas constant. The forward reaction coefficient was used in this investigation as a first step analysis of the effect of the thermal boundary layers and non-adiabatic boundary conditions on the observed temperature fields.
The boundary conditions are as follows:

For the Navier-Stokes Equations, the no slip condition is applied at the walls since the fluid is basically liquid water in our studies. At \( z = -50 \mu m \), a uniform velocity field, \( V_z \), normal to the xy-plane is introduced. The following correlation was used to check if the entry length (-50µm<z<0 µm) is sufficient for velocity profile full develop at the inlet plane (z=0µm) [81]:

\[
\frac{L_{ent}}{D_{hyd}} \approx \frac{0.6}{1 + 0.035 \cdot Re_{hyd}} + 0.056 \cdot Re_{hyd}
\]  

(3.20)

In Eq. (3.20), Reynolds number is defined as the ratio of inertial forces to viscous forces and can be calculated with the relation \( Re_{hyd} = D_{hyd}V_z/\nu \). \( D_{hyd} \) is the hydraulic diameter of the flow channel (4A_{xy}/Perimeter), \( V_z \) is the average velocity and \( \nu \) is the kinematic viscosity. Using the half width value for the inlet channels, entrance length was calculated as 12µm and 11µm for \( V_z = 0.05 \text{ m/s} \) and for \( V_z = 0.005 \text{ m/s} \) respectively.

The development of the velocity profile after the two channels merge is shown in Figure 3.19. The velocity profile has a stagnation point at \( x = w/2 \) at \( z = 0 \mu m \), when the two streams meet, and becomes fully developed before \( z = 50 \mu m \). Higher velocity near the outlet walls in the inlet region can be attributed to the sudden decrease in the wall shear stress caused by the change in the channel wall geometry. This behavior of velocity increase in the channel walls near the inlet was also reported by Kamholz et al. [82] and Beard et al. [21] in their studies including modeling mass diffusion in T-sensor configuration. The uniform velocity profile was imposed in each inlet channel, because the velocity in the z-direction (along the channel length) is uniform across x-direction, but is a function of y, as numerically shown by Yager [15].

\[
V_z(x, y, z = -50\mu m) = V_{inlet}
\]  

(3.21)
For the energy boundary conditions, the upper surface of the PDMS is exposed to convection to the ambient where, $T_{amb} = 298.15 \, K$ and a heat transfer coefficient of $h = 18 \, \frac{w}{m^2 \cdot K}$.

\[
-k \frac{\partial T(x, y = L_y, z)}{\partial y} = h[T(x, y = L_y, z) - T_{amb}]
\]  

(3.24)

Figure 3.19: Velocity profile development after the two developed flows merge at the mid-plane, $y = d/2$ for the case $V_z = 0.005 \, m/s$. 
To calculate the heat transfer coefficient, Nusselt number (Nu) was estimated using the relation for flat plates [83],

\[
Nu_L = \frac{h \cdot L_{PDMS}}{k_{air}} = 0.664 \cdot Re_L^{1/2} \cdot Pr^{1/3} = 1.89 \tag{3.25}
\]

In this calculation above, characteristics length was calculated as

\[
L_{PDMS} = \frac{Area}{Perimeter} \quad \text{where area and the perimeter corresponds to the top surface of the PDMS surface facing ambient air.}
\]

\[
T(x,y = -0.670\text{mm},z) = T_{set} = 298.15\text{K} \tag{3.26}
\]

The bottom surface of the glass chip was set to uniform temperature value, \(T_{set}\), similar to the experimental conditions where the glass chip sits on a temperature regulated surface.

\[
\frac{\partial T(x = 0,y,z)}{\partial x} = 0 \tag{3.27}
\]

\[
T(x,y,z = -50\mu\text{m}) = T_{inlet} = T_{\text{amb}} \tag{3.28}
\]

The boundary conditions for the mass flow are as follows, the reactants are introduced from two different inlets and merge at \(z = 0\ \mu\text{m}\), where mass diffusion starts:

\[
[C_j](x,y,z = -50\mu\text{m}) = M_j \tag{3.29}
\]

where, \(M\) is the molar concentration of species \(j\).

All of the channel walls are impermeable such that,

\[
\frac{\partial [C_j](x,y,z)}{\partial x} \bigg|_{x=0,w} = 0 \tag{3.30}
\]

\[
\frac{\partial [C_j](x,y,z)}{\partial y} \bigg|_{y=0,d} = 0 \tag{3.31}
\]
And the mass balance applied at the inlet and the outlet yields:

\[ \dot{m}_{\text{inlet}} = \dot{m}_{\text{outlet}} \quad (3.32) \]

These boundary conditions correspond to a configuration where the microfluidic channel is on a nearly isothermal heat sink. Sen et al. [78] numerically designed an isothermal delivery system to achieve such conditions. The justification of adiabatic boundaries at the channel sidewalls are: 1) the thickness of the insulative PDMS layer in x-direction is much larger than that in the y-direction (normal to the glass surface), 2) Since \( w \gg d \) (Figure 2), the area of the upper and lower channel walls, \( A_{xy} \) is greater than the area of the side channel walls, \( A_{yz} \) (\( A_{xy} \gg A_{yz} \)); 3) Velocity in the x-direction is zero. The effect of gravity is neglected. The laminar flow approximation is justified by the low Reynolds numbers (Re). Using the properties of liquid water at 298.15 K, channel dimensions and velocity values provided above, \( \text{Re}_{\text{hyd}} = 0.091 \) for \( V_z = 0.005 \frac{m}{s} \) and \( \text{Re}_{\text{hyd}} = 0.905 \) for \( V_z = 0.05 \frac{m}{s} \). Since water is the most abundant species in the mixture and the mass fraction of the other species is small, and the overall temperature differences are within \( \pm 3K \), the thermophysical properties of the water at \( T = 298.15 \) K are used to represent the properties of the liquid solution. Using these values Peclet values were calculated as \( \text{Pe}_T = 6.72, \text{Pe}_M = 490.20 \) for \( V_z = 0.05 \frac{m}{s} \), and \( \text{Pe}_T = 0.67, \text{Pe}_M = 49.02 \) for \( V_z = 0.005 \frac{m}{s} \).

The above transport model can be solved for the temperature and concentration fields in the device shown in Figure 1.7 and Figure 3.18. As a specific test case inlet solutions of NaOH and HCl were used. As stated earlier a base-acid solution was used in the numerical model because it is commonly used in calorimeter to standardize devices. This reaction provides a known energy release that is well documented even though it is a dissociation reaction with an
enthalpy of dilution rather than an enthalpy of reaction. The reaction and associated energy release are a result of the dissociated \( \text{H}^+ / \text{H}_2\text{O}^+ \) ions reacting with the \( \text{OH}^- \) ions to form the product. For the purpose of the present numerical modeling and interpretation of the thermal and concentration fields the energy released which is included in the energy balance as an internal energy source and which equally well describes either the enthalpy dilution or enthalpy of reaction type of process. For purposes of numerically modeling and investigating the general trends in this type of problem, the molecular diffusion coefficient was used for this purpose. It was felt that the difference in the modeling results using this hypothetic reaction-diffusion model would be consistent with the differences one would expect using different reactants and reactions and consistent with the present investigation. The equation for the reaction is,

\[
\text{NaOH (aq)} + \text{HCl (aq)} \rightarrow \text{NaCl (aq)} + \text{H}_2\text{O}
\]  

(3.33)

Where NaOH corresponds to reactant A and HCL corresponds to reactant B in the generalized development. Water is considered as the common solvent.

At the inlets, \( z = -50\mu\text{m} \), two different streams with different mass fractions of species enter the channel. For \( 0 < x < w/2 \) a fixed concentration of HCl (1M or 6M) is applied. For \( w/2 < x < w \) a fixed concentration of NaOH (1M or 6M) is applied. These values are typical of reported concentrations used to benchmark calorimeter experiments [32, 34]. Concentration values of 1M and 6M were investigated to determine the effect of molarity on the temperature and concentration fields. Downstream the products and excess reactants exit the channel at conditions governed by the diffusion and reaction equation. The binary diffusivity constants of HCl and NaOH are set to a constant value of \( D = 2.0 \cdot 10^{-9} \frac{m^2}{s} \). This value is reported by Leist et al. [84] for mixtures of HCl and NaOH. The constants for the Arrhenius reaction rate equation
and the values for the enthalpy of formation data for each species are taken from the reported
values in the literature [85] and are reported in Table 3.2 and Table 3.3 for $T_{\text{ref}}=298.15\text{K}$.

Table 3.2: The constants used for the Arrhenius reaction rate equation.

<table>
<thead>
<tr>
<th>$A$</th>
<th>$E_a$ [J/kmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.69 \times 10^{11}$</td>
<td>$3.33 \times 10^{6}$</td>
</tr>
</tbody>
</table>

Table 3.3: Enthalpy of formation and molecular weight values of the species.

<table>
<thead>
<tr>
<th></th>
<th>$NaOH$</th>
<th>$HCl$</th>
<th>$NaCl$</th>
<th>$H_2O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta H_f^0$ [J/mol]</td>
<td>$-4.70 \cdot 10^{5}$</td>
<td>$-1.67 \cdot 10^{5}$</td>
<td>$-4.07 \cdot 10^{5}$</td>
<td>$-2.86 \cdot 10^{5}$</td>
</tr>
<tr>
<td>$M$ [kg/mol]</td>
<td>$40.00 \cdot 10^{-3}$</td>
<td>$36.46 \cdot 10^{-3}$</td>
<td>$58.44 \cdot 10^{-3}$</td>
<td>$18.02 \cdot 10^{-3}$</td>
</tr>
</tbody>
</table>

3.3.1.3. Validation

The convergence of the simulations is checked by evaluating the residuals; this value is
on the order of $1 \times 10^{-16}$ for our runs. Also, mass and heat flux balances are checked upon
convergence to ensure continuity is satisfied in all of the equations.

Figure 3.20: Comparison of velocity profiles numerically computed by Fluent with
the analytical solution [81].
For validation of the flow field numerical results for the velocity magnitude are compared with the available analytical solution for Poiseuille flow between stationary parallel plates [81]. Normalized velocity fields obtained from several x and z regions are compared with the analytical solution and found in good agreement with the analytical velocity profile as shown in Figure 3.20.

Additionally, the temperature field obtained by the simulation is qualitatively compared to existing experimental data in the literature and presented in Figure 3.21. Pradere et al. measured the temperature field of a large substrate surrounding the microreactor, using an infrared camera (in digital units) [32]. The configuration [32] is a laminar coflow reaction of 0.25M NaOH and 0.25M HCl solutions with a flow rate of $1000 \mu l/h$. The domain size in z extends to 40 mm.

Figure 3.21: Comparison of the temperature field obtained from the numerical simulation (right) at the surface $y=0$ µm, to the experimentally measured temperature field by Pradere et al. (left) [32].
Figure 3.22: Concentration and temperature profiles along the channel width at z=300µm for different grid spacing values.

Since our domain size is 5 mm, in the comparison simulation the flow rate was decreased to $90 \mu l/h$. The behavior of the temperature contours is qualitatively similar, though the resolution and scale of the compared domains are different.

Grid convergence analysis was performed at three different node spacing values. The results were presented in Figure 3.22. No significant change was observed in the concentration and temperature profiles beyond 500000 nodes.

The convergence of residual values for each calculated value in the simulation was presented in Figure 3.23. Convergence was achieved after approximately 5500 iterations in all simulations.
3.3.1.4. Simulation Results and Discussion

The simulation was used to investigate how the thermal boundary layer affected heat transfer at the upper plate and to calculate the development of the temperature and concentration fields for different velocities and molarities. Additionally, the temperature profile across the thin dimension, the channel height, was examined to determine the difference between the temperature and concentration at the channel floor to that of the bulk of the fluid.

Formations of the temperature and concentration fields are presented in Figure 3.25 and Figure 3.26, respectively. The values shown are at y=0 (the channel floor) and inlet concentrations of 1M HCl and 1M NaOH. The solutions from two simulations with different
flow rates \( Q = 25 \frac{nl}{s} \) and \( Q = 250 \frac{nl}{s} \) are shown. Also shown in Figure 3.25 are the results for 6M inlet solutions. For comparison, a case with adiabatic boundary conditions on the upper and lower plates (y-direction) is also presented in Figure 3.25f.

![Normalized temperature profiles](image)

**Figure 3.24:** Normalized temperature profiles for 1M concentration, \( V_x=0.5\text{m/s} \), 6M concentration, \( V_x=0.5\text{m/s} \).

Figure 3.25 confirms that heat transfer in y-direction changes the nature of the problem significantly. In the adiabatic case the temperature increases downstream. In contrast, for the non-adiabatic cases, the system forces the temperature field to the equilibrium temperature of the surroundings. As seen in Figure 3.25f, the adiabatic case, the temperature of the fluid in the device increases to a uniform value that is above that of the surroundings. All of the energy released by the reaction leaves the system in the non-adiabatic cases. While the colored contours plots provide a good overview of the temperature field, the line plots are shown to provide clarity.
when the affected region is small. As seen from the combinations of a and b in Figure 3.25, the spatial effect of the source term is dependent on the velocity with the lower velocity producing a smaller affected region than the higher velocity case. Comparing the results in Figure 3.25e for the 6M solution to that of Figure 3.25c and Figure 3.25d illustrates that higher concentrations yield more energy and higher temperatures, but the basic profile of the temperature profile remains the same as seen on Figure 3.24. As discussed in Section 3, this information can be used to determine the preferable locations of sensors in an experimental device (i.e. temperature and concentration sensor spacing in z and x).

**Figure 3.25**: (a) Temperature contours for 1M concentration, Q = 25nL/min, (b) corresponding line plot for (a), (c) Temperature contours for 1M concentration, Q = 250nL/min, (d) corresponding line plot for (c), (e) Temperature contours for 6M concentration, Q = 250nL/min, (f) Temperature contours for 6M concentration, Q = 250nL/min, adiabatic case.
Comparing Figure 3.25 and Figure 3.26 shows the difference between the temperature field and the concentration field of the reaction product. The extent of diffusion in x increases in the downstream region, which would correspond to a higher concentration signal of reaction...
product in experimental measurements. For the temperature field, the change due to the reaction occurs near the inlet region.

Figure 3.26 shows the concentration distribution for the product (NaCl for this reaction). Comparing Figure 3.26 (a) and (c) shows that increasing the flowrate (or velocity) decreases the width of the diffusion region in x-direction. This result suggests that, for concentration measurements, resolution in x-direction can be adjusted by changing the flowrate.

The similarity of mass and thermal transport is seen in this comparison as the concentration profiles more closely resemble the adiabatic thermal case, Figure 3.25 (f), than the temperature profiles for the non-adiabatic cases, Figure 3.26 (a), (b) and (c).

3.3.1.5. **Thermal Boundary Analysis in the Flow Domain**

The thermal boundary effects were analyzed in a smaller domain to study the temperature development in the y-direction (normal to the glass surface). Figure 3.25 shows the contours of the temperature development in the entrance region caused by the spatially non-uniform energy source due to the reaction. Hence, for the thermal boundary analysis, temperature profiles are taken from the regions close to the half-width distances in x, since highest temperature change occurs here due to the laminar diffusion interface in the center of the channel.

Surface based sensors are expected to measure values in a region only 100-300nm above the flow surface (y=0, the plane where sensors are present for NHA sensors) [20] or through the external surface interface (i.e. through glass or PDMS layers for thermography based measurements). If such sensors are to be used to measure reaction properties than the observed surface temperature and concentration values must accurately represent the respective fields in bulk of the fluid. The simulation results are used to estimate the deviation between the
temperature at the surface layer, close to the channel floor, and the mean channel temperature in y-direction. Figure 3.27, Figure 3.28 and Figure 3.29 show the results of this investigation, plotting temperature across the channel thickness at various locations.

**Figure 3.27:** Development of the temperature profiles at x=w/2 (M=1.0).

**Figure 3.28:** Development of the temperature profiles at x=w/2 (M=6.0).
To calculate the agreement between the sensed temperatures from the mean channel temperature the following equation is used:

$$\text{Agreement(\%)} = \frac{1}{L_{\text{SPR}}} \int_0^{L_{\text{SPR}}} [T(y) - T_{\text{set}}] \, dy \cdot 100$$

In the equation above, $L_{\text{SPR}}$ represents the distance normal to the $y=0$ surface where the detection occurs by the surface plasmon resonance (SPR) based sensors. This distance is governed by the wavelength of the incident light [20] (632nm in our research). This integration was done numerically for several $x$ and $z$ regions. The smallest agreement values were 98.61% for the low molarity case, and 98.58% for the high molarity case. This result suggests that a temperature field measured by surface sensors covering the flow surface accurately represents the average temperature of the channel.
3.3.1.6. **Heat Loss Analysis**

The heat transfer on the lower and upper surfaces of the flow surface was analyzed in order to understand the relation between these energy transfer mechanisms as compared to that convective with the flow. As discussed in the introduction section, one significant difference between the thermal and mass transport problem is the non-adiabatic boundary conditions and its effect on the temperature field. This analysis leads to simplifications which can be used for practical calculations such as determining the heat loss calibration constant. For the simulation with \( V_z = 0.005 \text{ m/s} \), the heat flux in the \( z \) direction, along the channel length at different \( x \) values was calculated. The laminar diffusion interface, \( x = 250 \mu\text{m} \) corresponds to the mid-plane of the channel. The results for both \( y = 0 \mu\text{m} \) (glass chip surface) and \( y = 10 \mu\text{m} \) (upper PDMS surface) are shown. Figure 3.30 shows the heat fluxes on the top and bottom surfaces of the flow channel at different \( x \)-positions. The dashes lines represent the heat fluxes on the top insulative PDMS surface, and the lines with the remaining lines represent the heat flux on the bottom surface. Quantitative comparison between two heat fluxes was performed to get a clear understanding of contribution of each quantity for the surface measurement approach. The results showed that heat flux from the \( y = 0 \) surface across the channel is larger compared to the insulative PDMS surface at \( y = 10 \mu\text{m} \).

At any \( x \) location this ratio is calculated as:

\[
\frac{\text{Heat Flux Upper Surface (x line)}}{\text{Heat Flux Lower Surface (xline)}} = \frac{\min(q''_{y=10\mu\text{m}})}{\min(q''_{y=0\mu\text{m}})} \cdot 100 \tag{3.35}
\]

Using Eq. (3.35), the heat loss from the upper surface at \( x = 250 \mu\text{m} \) equals 3.6% of the lower heat flux. The same calculation can be done by calculating the total heat flux over the entire lower and upper surface, this value is calculated from the Fluent using:
This value is calculated as 0.7% using the values obtained from Fluent.

\[
\frac{\text{Heat Flux Upper Surface (Whole Area)}}{\text{Heat Flux Lower Surface (Whole Area)}} = \frac{q''_{y=0\mu m}}{q''_{y=10\mu m}} \cdot 100
\] (3.36)

Figure 3.30: Heat flux values at different x values along the (flow) z direction at the lower and upper surfaces. There is sufficiently low heat flux through the PDMS surface that the traces are nearly indistinguishable for all positions across the channel (dashed lines, top of the plot).

The same conclusion on heat transfer for the lower and upper surfaces can be also derived by looking at Figure 3.27 to Figure 3.29. The slope of the temperature lines are close to zero at y=10\mu m compared to the slope of the temperature lines at y=0\mu m. This is an expected result based on the boundary conditions applied to the bottom and top surfaces, but it gives a
quantitative comparison between two heat fluxes to see if the heat loss is negligible for the surface measurement techniques and the development of analytical calculations.

### 3.3.1.7. Discrete Sensor Positioning Analysis

In this section, the analytical methodology developed previously was applied to the temperature, concentration and heat loss maps at the y = 0 µm surface. The enthalpy of reaction reference value was used as the benchmark. The spacing between the calculation nodes in x and z directions were increased to analyze the impact of sensor spacing on measurement accuracy. The spacing range used was appropriate for a nanohole array application mapping a reaction zone with a coflow configuration. This analysis is important since spacing between each nanohole array sensor is limited due to the interference effects between the sensors and also due to the material constraints.

For the sensor spacing analysis, temperature, heat flux and concentration at y=0 zero surface are taken from Fluent. Then, each term in Eq. (2.9) is calculated using equally spaced points at different spacing values and enthalpy of formation value is estimated. MATLAB is used for approximating first order derivatives using first order finite differencing schemes and double integration term in Eq. (2.8). Using this formulation enthalpy of reaction was estimated. This procedure is repeated using different values of spacing between data points at different inlet velocities and different intervals in z-direction (flow direction).

The surface plot in Figure 3.31 shows the heat loss coefficient H(x,z) at y = 0 surface with all the nodes included for 0.15 µL/min flow rate. Each black dot represents a sensor and data is taken only from these locations to approximate the terms in Eq. (2.9) to calculate the
enthalpy of reaction. In this case sensors are equally spaced in x and z directions at a distance of 30 μm.

Figure 3.31: Surface plots of mass fraction of NaCl and the heat loss coefficient $H(x,z)$ [W/m$^2$.K] for $Q = 0.15μL/min$ case. Each dot represents a sensor (shown here 30 μm spaced, sensor diameter not to scale) taking local information.

For the sensor positioning algorithm, three different approaches were used: (i) for sensor spacing $r$, place a sensor at $x=r, 2r, 3r...$ for all available sites within the channel, (ii) place a sensor at $x=w/2, (w/2) ±r, w/2±2r, ...$, and (iii) place a sensor at $x=(w/2)±(r/2), (w/2)±3(r/2), (w/2)±5(r/2)$. Note that (i) is not necessarily symmetric about channel y-z midplane.

The analysis was applied to three different flow rates, 0.15 μL/min, 1.5 μL/min, 15 μL/min. In our geometry, these flow rates correspond to velocities 0.0005 m/s, 0.005 m/s, 0.05 m/s respectively. Enthalpy of reaction was calculated in each of these simulation cases by taking values only at discrete locations which correspond to NHA sites. These calculations serve as the
fundamentals of the calorimetric calculations in T-Sensor configuration with a sensor measuring values close to the flow surface. Figure 3.32 shows the deviation of the calculated value from the actual value of the enthalpy of the reaction (56 kJ/moles for this specific reaction). For this calculation, the domain extends from \( z=200 \) to \( 800\mu m \) and \( x \) domain extends from \( x=0 \) to \( 500 \mu m \) and sensors are placed from the center (algorithm (ii), above). Figure 3.32 shows that the error is highly dependent on the flow rate. For the 0.15 \( \mu L/min \) flow rate, after 30\( \mu m \) spacing, the error is larger than 10\%. For the 1.5 \( \mu L/min \) flow rate, calculations start to diverge for sensor spacing larger than 16 \( \mu m \) in \( x \) and \( z \) directions. For the 15 \( \mu L/min \) flow rate, the error starts to accumulate for node spacing larger than 2 \( \mu m \) spacing.

![Figure 3.32](image)

**Figure 3.32:** Deviation \( \% \) of the estimated enthalpy value using Eq. (2.9) from the actual value at different sensor spacing values for different flow rates.
Based on Figure 3.32, it can be concluded that greater resolution is required for high flow rates. This is due to the smaller diffusion width in x-direction due to the low residence time. Limited diffusion width (in x-direction) also causes temperature changes to occur in a narrower region. It should be also noted that, these results are dependent on the diffusivities of the species of the reaction. Velocity can be adjusted to achieve necessary resolution depending on the reaction diffusivity. It should be noted that, increasing the velocity (or the flow rate) increases the span of the gradients in z direction but lowers the span of the gradients in x direction (low resolution is required in z direction, high resolution is required in x direction). In addition, decreasing the velocity (or the flow rate) decreases the span of the gradients in z direction but increases the span of the gradients in x direction (high resolution is required in z direction, low resolution is required in x direction).

![Figure 3.33: Deviation % of the estimated enthalpy value using Eq. (2.9) from the actual value at different sensor spacing values for 0.15 μL/min flow rate using different sensor spacing approaches.](image-url)
Next, the dependence of sensor positioning (at fixed spacing) on the estimation is studied. Three sensor placing approaches were tested for the 0.15 μL/min flow rate case and the enthalpy value estimated using Eq. (2.9). Figure 3.33 shows the results for three different sensor placement approaches. It can be observed that after 40 μm spacing, a fluctuation in the deviation starts to occur. This is more evident for the case where sensors are placed with reference to the wall at x=0μm (method (i). The analysis suggests that this is due to the shape of the concentration, temperature and the heat loss coefficient curves as functions of x (which are similar to a Gaussian distribution). These functions are presented in Figure 3.34.

Figure 3.34 indicates that the width of the concentration curve is narrower (in x-direction) compared to the temperature and heat loss curves. A 60μm spacing configuration can represent the heat loss and temperature averages across the width of the channel but not the mass fraction at the regions near the inlet. This behavior is due to the low values of mass diffusivity coefficient in x direction compared to the larger values of thermal conductivity (diffusivity) constant.

At certain spacing configurations, however, sensor locations can fortuitously correspond to locations where a single point represents the total average of the curve (the vicinity of the horizontal red lines in the Figure 3.34). This behavior results in fluctuating behavior in the error term as seen in Figure 3.33. However, these locations are dependent on the curve characteristics and can change with many parameters such as the nature of reaction, and z-location. Hence, the error analysis should be done with extra caution. The results show that this behavior starts to occur after 40 μm spacing.
Figure 3.34: Plots of Heat loss coefficient, Temperature at z=50μm and mass fraction of NaCl at z=50μm and 800μm as a function of z. Red dots indicate the sensors spaced at 60μm starting from center. Horizontal red lines present the average values of the curves.

A similar analysis was repeated for the 0.15 μL/min flow rate case but with heat release estimated using Eq. (2.9). Here in this case, the mass fraction approximation is isolated from the calculations. This calculation was performed since the concentration curve behavior is harder to capture compared to other curves as shown in the Figure 3.34. The results are presented in Figure 3.35. Isolating mass fraction from the calculation decreases the fluctuation effect and heat release can be estimated within 10% error at the spacing of 50 μm.
Next, the effect of the signal noise on the calculations is analyzed. MATLAB is used to impose random deviation (+/- 0.01K for temperature, +/- 250 W/m²K) on each numerically calculated component before calculating the enthalpy of reaction. Figure 3.36 illustrates the effect of the imposed noise on the temperature and heat loss coefficient components.

Since the noise imposed is random within a given interval, several consecutive runs are performed. Figure 3.37 summarizes the results of 10 consecutive runs for Q = 0.15 μL/min using the z domain = [0,800 μm] and the complete x domain. For the noise imposed run tests, the center is taken as reference and first sensors are placed half the value of sensor spacing distance spaced from the center (approach iii). The average deviation in approximated enthalpy for these runs is close to 5%.
Figure 3.36: Plots of simulated noisy (a) temperature and (b) heat loss coefficient signals as a function of $x$ at $z = 200 \, \mu m$ intervals for $Q = 0.15 \, \mu L/min$ case.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.36.png}
\caption{Plots of simulated noisy (a) temperature and (b) heat loss coefficient signals as a function of $x$ at $z = 200 \, \mu m$ intervals for $Q = 0.15 \, \mu L/min$ case.}
\end{figure}

Figure 3.37: Calculated error in the enthalpy estimations of 10 consecutive runs using noisy data obtained from $Q = 0.15 \, \mu L/min$ flow rate. The sensor spacing is $30 \, \mu m$, and sensors are positioned half the sensor spacing value from the center.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.37.png}
\caption{Calculated error in the enthalpy estimations of 10 consecutive runs using noisy data obtained from $Q = 0.15 \, \mu L/min$ flow rate. The sensor spacing is $30 \, \mu m$, and sensors are positioned half the sensor spacing value from the center.}
\end{figure}
Finally each term in Eq. (2.8), (pg. 43) is individually calculated and compared with the minimum node spacing (2 μm) values to analyze the error contribution of each component in Eqn. (9). This analysis is done for the Q = 0.15 μL/min flow rate and sensors are placed starting from the center. Each term in Eq. (2.8) can be explained as follows:

\[
\text{HeatRelease} = \text{Heat Loss Normal to the Chip Surface} + \text{Thermal Bulk Flow} + \text{Thermal Conduction in the Flow Direction}
\]  

(3.37)

The results are presented in Figure 3.38. Individual contributions of each term in Eq. (2.8), (pg. 43) are plotted for different sensor spacing values. The 2 μm spacing case is taken as the reference value. The results suggest that, in all spacing values, the error is dominated by the conduction term. This term includes the first order derivative in the z-direction. This is expected since finite differencing is highly dependent on node spacing and error increases as the node spacing increases.
spacing increases. It is important to note that the conduction and bulk flow thermal terms are only calculated at the inlet and the outlet planes of the calculation interval. The heat loss term covers the entire calculation area since integration is performed across the flow surface. Following this thought, the sensor spacing should be decreased at the inlet and outlet regions of the calculation region since they are estimated only at certain z locations.

### 3.3.2. Effects of the Light Source and the Flow Cell for NHA Sensors

In this section, we investigated effects of different excitation (light) sources and flowcell configurations (material and assembly layers) on the EOT signal quality with respect to temperature changes. Current literature discusses pressure induced deformations and related interferences in an optical setup. Our research discusses effect of temperature and thermo-mechanical changes on the interference patterns (due to either to the light source, flowcell material or both) and on the EOT signal. These optical interference patterns have a highly nonlinear dependence on the temperature changes which negatively impacts EOT-temperature calibrations in a manner that appears as noisy signal component in the measurements. Temperature dependent EOT measurements were taken using different optical setups and flowcell assembly configurations. Finally, we present EOT-temperature calibrations performed for liquids with different index of refraction values in an interference-free design which minimizes the noise to signal ratio. These calibrations provide experimental support that the observed transmitted signal is related to the dielectric constant induced EOT resonance that is the foundation of the proposed sensor.

A list of the experiments with different components is listed in Table 3.4. The last column in this table indicates if the optical setup results in an image with interference reflections in the signal.
Two different flowcell designs were tested. The first design had a 2mm thick PDMS in the light path and has a channel height of 10μm. The second design had the same flow channel geometry and PDMS was used as a 100μm thick gasket between the glass cover and sensing chip. The second design had no PDMS in the optical path. The two different configurations are illustrated in Figure 3.39.

Figure 3.39: Cross sectional view of the different flowcell designs, (a) 2mm thick PDMS with 10μm thick flow channel, (b) 100μm PDMS gasket.

Several optical configurations were tested to isolate the fringe pattern source. These configurations are presented in Table 3.4 in detail. For each configuration, the temperature was increased from 28ºC to 36ºC and brought back to 28ºC in steps of 2ºC increments in 15 minutes intervals. This amount of time ensured the system to reach steady state conditions.
Table 3.4: List of optical configurations tested. Bare chip means no flowcell was attached on the sensing chip. Tests 9, 10, 11 and 13 were performed with both air (empty) and water in the flowcell channel.

<table>
<thead>
<tr>
<th>Test No.</th>
<th>OPTICAL LINE</th>
<th>Fringes present?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LED</td>
<td>Polarizer</td>
</tr>
<tr>
<td>2</td>
<td>LED</td>
<td>Polarizer</td>
</tr>
<tr>
<td>3</td>
<td>Laser</td>
<td>Polarizer</td>
</tr>
<tr>
<td>4</td>
<td>Laser</td>
<td>Diffuser</td>
</tr>
<tr>
<td>5</td>
<td>Laser</td>
<td>Diffuser</td>
</tr>
<tr>
<td>6</td>
<td>Laser</td>
<td>Polarizer</td>
</tr>
<tr>
<td>8</td>
<td>Laser</td>
<td>Diffuser</td>
</tr>
<tr>
<td>9</td>
<td>Laser</td>
<td>Diffuser</td>
</tr>
<tr>
<td>10</td>
<td>Laser</td>
<td>Diffuser</td>
</tr>
<tr>
<td>11</td>
<td>LED</td>
<td>Polarizer</td>
</tr>
<tr>
<td>12</td>
<td>LED</td>
<td>Polarizer</td>
</tr>
<tr>
<td>13</td>
<td>LED</td>
<td>Polarizer</td>
</tr>
</tbody>
</table>
The chip used in this experiment had 19 colinear NHAs in two rows across the channel width. Each NHA consisted of 10x10 array of 150nm diameter nanoholes with pitch distance of 350nm. The spacing between each NHA was 30μm. The EOT at all sites was monitored simultaneously using the CCD camera and recorded using LabVIEW software. The mean value of the signal from each NHA site was taken over the time at thermal equilibrium. LabVIEW software was used to average over the pixels covering each NHA sensor site. In our setup, a NHA sensor site covered an area of approximately 13x13 pixel zone. Averaging over the pixels and the size of the averaging area are described in detail in Section 3.3.3. This approach saves both memory space and speed since instead of capturing entire image, only regions of interest were saved to the data file. A CCD image of a single NHA site and the averaging region are shown in Figure 3.40.

Figure 3.40: CCD image of the transmission from a single NHA sensor. The squares represent different averaging regions, blue: 3x3, yellow: 7x7 and red: 13x13.
At the steady state regions, 250 data points were used to calculate the EOT signal for corresponding temperature. During the EOT-temperature calibration experiments, the exposure of the CCD camera was adjusted such that maximum intensity values from the NHA sensors were below the saturation value. To observe fringes, high exposure times were used to make interference patterns visible. At low exposure rates, the fringes were not visible but affected the data. Our setup with the thin metal film allowed us to take such measurements in order to focus on the noise producing fringes and to understand their source.

In addition to the steady results, transient analysis of the EOT signal with respect to temperature change was performed. To do this, movies and images of the entire flow channel were captured in addition to the EOT signal from the NHA sensors.

Finally, two different liquids were injected consecutively to the fringe-free optical setup and temperature-EOT calibration tests were performed for DI water and ethanol. A syringe pump (Harvard Apparatus) was used for injection at a rate of 30µL/min.

![Image with Laser](Image with Laser)
![Image with Red LED](Image with Red LED)

**Figure 3.41:** CCD images of NHAs with PDMS flowchannel (a) with laser illumination, (b) with LED illumination.
Laser or LED light sources were used to illuminate the chip assembly. High exposure setting was used to observe fringes. CCD images of the entire flow channel were captured in both illumination configurations. These patterns are shown in Figure 3.41 (a) as diagonal black and white bands. This primary interference pattern was avoided by using an incoherent light source, such as LED. The improvement in the image is presented in Figure 3.41 (b). This behavior is consistent with the observations of Wilkop and O’Brien et al. [75-76].

Images in Figure 3.42 were taken from a movie as the temperature of the laser-illuminated PDMS flowcell was varied. The flow channel can be identified by the two thin black horizontal lines that appear in the top and bottom regions of the images. Two different types of interference patterns can be observed. The first one is the diagonal bands. The direction of these bands is dependent on the clamping pressure as well as temperature. The change in the clamping pressure causes a change in the distance and relative angle between the reflective gold surface and the glass cover. We concluded that these fringes are due to the coherent light reflections between the gold and an upper interface (e.g., air—glass interface) as discussed in the introduction and are similar to those encountered in a Fabry-Perot device. The second sets of patterns are irregularly shaped and were inside the flow channel. We attribute these to the deformation of the flow channel under clamping pressure (fringes with curvatures inside the channel). When the controlled temperature was increased from 28ºC to 30ºC, both fringe patterns move during the temperature transient range. The fringe movement stopped when thermal equilibrium was reached. To illustrate movement, a single band was highlighted and its position was shown at different times/temperatures in Figure 3.42 during a temperature change. The movement of the fringes was not observed during isothermal injection of different liquids and is highly dependent on the temperature changes.
Figure 3.42: Effect of the temperature on the fringe patterns, $T_1 < T_2 < T_3 < T_4$ at times $t_1$, $t_2$, $t_3$, $t_4$ with laser as the light source and with PDMS flowcell. Dashed yellow line highlights the movement of a specific interference pattern.
Note that in this system, the fringes are observed by focusing on the back side of the gold film, relative to the direction of light incidence. The camera is detecting light that is transmitted through the thin film gold and the glass substrate.

Figure 3.43: CCD images of NHAs with PDMS flowchannel and LED light source (a) with the condenser lens, (b) without the condenser lens, (c) Intensity profiles across the center of the images. Each color in plot (c) corresponds to the intensity variation along the lines with the same color in (a) and (b). Green and red are for the test without the condenser while black and blue are with the condenser in the optical path.

Having established that interference fringes are suppressed using the incoherent light source, the collimated LED was tested for measurement performance. The collimated LED source used in our setup had a Gaussian intensity profile. Spatially uniform illumination is
important for NHA sensors since the EOT signal is proportional to the incident light. Figure 3.43 shows the comparison of two illuminations, with and without a condenser lens. The intensity across the center of the images were plotted to illustrate the distribution of Gaussian intensity profile (bright spot) of the collimated LED. Using a condenser lens between the collimated light source and the NHA chip converted the Gaussian profile to a uniform intensity profile, and also increased the intensity level by approximately 2.5 times that of the original beam in regions away from the center.

Figure 3.44 shows the interference patterns due to the PDMS channel deformation when a LED light source was used. Fringe patterns were roughly aligned to the flow direction since the deformation is longitudinal (similar to a half cylinder shape). At different clamping pressures, interference patterns changed as illustrated in Figure 3.44. Similar behavior was observed in the case of a temperature change. A temperature change resulted in thermo-mechanical stress in the clamped PDMS flowcell assembly. The induced stress led to change in the deformation shape of the upper wall of the flow channel. Even small intensity changes due to deformation affect the EOT signal.

Due to the resonating nature of the EOT signal itself, and its proportional dependence on the incident intensity, it would be erroneous to digitally filter-out both interference patterns. Relative orientation between the propagating wave and the NHA position effects the EOT behavior [86]. The characteristic of the propagating wave is dependent on polarization, wavelength, and the incident intensity (and angle) [49]. Hence non-uniformity in the incident intensity can cause undesired effects on the EOT signal. The solution is to eliminate or significantly reduce the magnitude of the fringes. Our setup and the camera settings allowed the
fringes to be visible to observe their behavior. In a normal operation (low exposure), fringes contribute to EOT signal as the noise source.

Figure 3.44: Effect of pressure on the fringe patterns inside the flow channel at isothermal conditions. $P_1<P_2<P_3<P_4$. 
To reduce the impact of the upper wall of the flowchannel sagging, PDMS flowcells with different channel heights were tested (50 to 100µm thick). Increasing height/width ratio reduced but did not eliminate the problem. A gasket type flow cell design with a glass cover top was used to remove the PDMS from the optical path since the glass is stiffer than the PDMS polymer. This design prevents sagging since only glass comprises the upper wall of the flow channel. The same flow channel design was used but channel height was increased from 10µm to 100µm in the gasket design. In this configuration, no PDMS was present in the illumination path. This PDMS gasket and glass cover flowcell design was tested with laser and LED. For the laser light source constructive/destructive interference patterns were present throughout the chip due to the reflections between the gold surface and top glass cover layer. These fringes were dependent on temperature and moved as the temperature was changed. Temperature-EOT calibration tests were performed for both LED and laser light sources. Raw EOT signal during a temperature change is shown in Figure 3.45. In both light sources, EOT signal was steady during thermally stable conditions (before and after temperature change). However, in the case of a temperature change, EOT signal fluctuated significantly during the temperature transient region. This behavior was due to the fringe movement over the NHA sensors for the laser light source. The intensity change over the NHA sensors directly affects the EOT signal. Using this configuration, results of an EOT–temperature calibration experiment with water for one sensor is shown in Figure 3.46. For the reasons explained above, the EOT temperature cross plot in Figure 3.46 (c) had a non-repeatable and non-linear behavior. This type of trend is not acceptable for a measurement device.

The PDMS gasket with a cover glass illuminated with an LED light source resulted in interference-free EOT signal during a temperature change as seen in Figure 3.45 (red line). The
change in the EOT signal is linear during the temperature transients. There is also considerably less noise in the EOT signal with the LED compared to the laser. Tests with a PDMS flow cell with a channel height of approximately 100 µm, in which the LED light traveled through a PDMS layer, did produce fringe pattern. The elimination of the fringes were not due to the increased to the channel height, but to eliminating the deforming PDMS in the light path.

![Figure 3.45](image)

**Figure 3.45:** EOT response during steady and transient temperature for Laser/Glass/Gasket and LED/Glass/Gasket combinations. Temperature was changed from 28°C to 30°C. Dashed ellipse highlights the transient region.

Finally, using the interference-free configuration (LED light source, polarizer, condenser lens and PDMS gasket and glass cover), EOT-temperature calibration experiments were performed for water and ethanol. This configuration is listed as Test 012 in Table 3.4. Figure 3.47 (a) presents the raw EOT signal for the complete experiment. Difference in the index of refraction value between DI water ($n_{water}=1.333$) and ethanol ($n_{ethanol}=1.360$), respectively, caused a shift in the base signal value. Temperature changes (increments or decrements) in the
system caused a step change in the EOT signal. Figure 3.47 (b) shows the history of the controlled temperature.

Green and red circles show where EOT data was averaged at each temperature. Figure 3.47 (c) shows the plot of EOT vs. temperature for DI water. Black lines are the plots for increasing temperature and red lines are the plots for decreasing temperature. Figure 3.47 (d) shows similar calibration plot for ethanol. Figure 3.47 (e) shows the normalized plots of the calibration for water and ethanol. A linear behavior was observed in the EOT signal when the temperature was changed.

Figure 3.46: (a) Raw EOT signal of a temperature calibration experiment for water with the laser, glass cover and PDMS gasket configuration, (b) Controlled unit temperature, (c) EOT vs. temperature cross plot.
Figure 3.47: (a) Raw EOT signal of a temperature calibration experiment for water and ethanol, (b) Controlled unit temperature, averaged EOT intensity values at different temperatures (c) for water (d) ethanol (e) Normalized intensity value for water and ethanol.
Figure 3.47 (a) also shows that the overall gain in the EOT signal due to temperature change for the ethanol is higher than the DI water. The ratio of the slopes of curves in Figure 3.47 (c) and (d) can be calculated as:

$$\frac{\text{slope}_{\text{ethanol}}}{\text{slope}_{\text{water}}} = \frac{(928 - 848)\text{CCDUnits}}{(36 - 28)\text{°C}} = \frac{(579 - 558)\text{CCDUnits}}{(36 - 28)\text{°C}} = 3.810$$ (3.38)

EOT signal is dependent on the change of the dielectric constant of the medium above the gold film. Dielectric constant is dependent on pressure, temperature, concentration of the medium and volumetric expansion coefficient. The difference of temperature dependence of EOT signal for two liquids can be explained due to the difference in the volumetric expansion coefficient difference between ethanol and water. Volumetric expansion coefficient for water and ethanol are $\beta_{\text{water}} = 207 \cdot 10^{-6}/\text{°C}$ and $\beta_{\text{ethanol}} = 750 \cdot 10^{-6}/\text{°C}$ respectively (at 20°C).

Kowalski et al. [56] related the temperature and pressure dependence of the dielectric constant in the sensing region, $\varepsilon_1$:

$$\varepsilon_1 = \left[1 + 2C_0\rho_0 \Phi \right] \left[1 - C_0\rho_0 \Phi \right]$$ (3.39)

where, $\Phi = \exp[\beta(T - T_0) - k_T(P - P_0)]$, $T_0$, $P_0$ are reference values for temperature and pressure, $k_T$ is isothermal compressibility factor, $\beta$ is the volumetric expansion coefficient, $n$ is the index of refraction, $C_0 = [(n_0^2 - 1)/(n_0^2 + 2)](1/\rho_0)$, $\rho$ is the density, $n_0$ and $\rho_0$ at the reference temperature conditions.
Taking the derivate of Eq. (3.39) with respect to temperature gives:

\[
\frac{d\varepsilon_\perp}{dT} = C_0 \rho_0 \beta \Phi \left\{ \frac{3}{[1 - C_0 \rho_0 \Phi]^2} \right\}
\]  

(3.40)

Assuming negligible pressure difference during no flow inside the flowcell, the \(k_T(P - P_0)\) term goes to zero in all terms. Using the physical values of water at 25°C as the reference values and substituting \(\beta_{\text{ethanol}}\) in Eq. (3.40) gives \(\left.\frac{d\varepsilon_\perp}{dT}\right|_{\text{ethanol}} = 7.403 \cdot 10^{-4} \frac{1}{\degree C}\). Substituting \(\beta_{\text{water}}\) in Eq. (3.40) gives \(\left.\frac{d\varepsilon_\perp}{dT}\right|_{\text{water}} = 2.030 \cdot 10^{-4} \frac{1}{\degree C}\).

The ratio of the dielectric change with respect to temperature in water and ethanol is,

\[
\frac{\left.\frac{d\varepsilon_\perp}{dT}\right|_{\text{ethanol}}}{\left.\frac{d\varepsilon_\perp}{dT}\right|_{\text{water}}} = 3.647.
\]

This value is in within 4.47% of the experimentally observed slope ratio in EOT vs. temperature plots of water and ethanol, which equals 3.810. This comparison suggests that case 012 of Table 3.4 yields an experimental design free of fringe related noise and that temperature dependency of EOT signal is being observed.

An interference-free optical configuration was developed for localized temperature measurements in microfluidic flow channels using NHA sensors. It was found that, a coherent light source creates interference patterns due to the reflections between the gold surface and top surface of the channel wall (glass or PDMS) and these interference patterns were sensitive to temperature and pressure. This interference can be avoided by using an incoherent light source. The deformation on the upper wall of the PDMS channel created Newton’s rings type structures even when using an incoherent light source. Using a gasket type design and using glass cover as the upper wall of the flow channel solved this interference problem. Using an optical
configuration with LED light source, a polarizer, a condenser and a flowcell with a glass cover and gasket design, EOT vs. temperature plots were created for DI water and ethanol. The change in EOT with respect to temperature was repeatable and linear. It was found that EOT signal gain due to temperature change (28 to 36°C in steps of 2°C) was 3.810 times higher than the DI water signal gain. This behavior was explained by the difference in the volumetric expansion coefficient, which is a factor in the change of dielectric constant due to temperature changes. This trend was also in agreement with the analytical relations.

3.3.3. Effect of Pixel Averaging Size over an NHA site

This section discusses the effect of the size of the intensity averaging region for an NHA site. CCD camera in the experimental setup used a 10x Nikon lens. Pixel size in the CCD sensor was 7.4µm x 7.4µm and the total number of pixels in the CCD camera was 2048 x 2048. The purpose of this investigation was to increase sensitivity by averaging over the regions with high intensity pixels. The 10x lens increased the magnification by 10 times and reduced the pixel size to 740nm. Considering that a 10x10 NHA with a pitch distance of 350nm had a size of 3.3µm, the footprint of a NHA sensor is 5 pixels. Due to the collimation of the light emitted from the NHAs the light transmission from an NHA site covered an area of 10x10 pixel region.

We investigated the pixel effect of averaging size on the repeatability of the EOT signal change due to temperature. For this series of experiments, LED light, PDMS gasket type flowcell with the glass cover was used. The flowcell was filled with water and temperature was increased from 28°C to 36°C in steps of 2°C in thirty minute intervals. During the thermal stable period (before changing temperature to the next set value) 100 consecutive images of the entire view field was recorded. This process was repeated at each set temperature, both during temperature ramp-up and ramp-down.
Next all recorded images were post processed in MATLAB. A MATLAB script was created to open each image, find the center pixel number of each NHA site and average around this pixel based on the desired averaging region size. The MATLAB script reported the averaged EOT values with respect to the set temperature, resulting in EOT vs. Temperature cross plot.

Figure 3.48 presents a CCD image of a single NHA site and the gray level pixels due to the EOT signal. The brightest pixel was considered as the center in the MATLAB script. The
squares with different colors represent the region where intensity average was taken. For this investigation, three different sizes were used, 3x3, 7x7 and 13x13.

Figure 3.49: Temperature calibration results using different averaging regions. (a) to (c) EOT vs. Temperature cross plots, black lines indicate increasing temperature, red lines indicate decreasing temperature. (d) to (f) raw EOT signals at steady temperature region. Each color represent a different set temperature.

Figure 3.49 and Figure 3.50 presents the results from the tests for different sensors (NHA sites). Plots on the top represent the raw EOT signal averaged over 100 images. Averaged EOT intensity values were cross plotted with respect to the set temperature. Since the transmitted light is brightest in the center and reduces to the background intensity level in Gaussian shape,
increasing the averaging region decreases the signal intensity value. Intensity value goes from 3500 CCD units to 1400 CCD units when the averaging region was increased from 3x3 to 7x7. This value reduced to 500 CCD units when 13x13 was used. Based on this conclusion, it can be advantageous to use an averaging region as small as possible to increase signal magnitude.

![Graphs showing temperature calibration results using different averaging regions.](image)

**Figure 3.50:** Temperature calibration results using different averaging regions. (a) to (c) EOT vs. Temperature cross plots, black lines indicate increasing temperature, red lines indicate decreasing temperature. (d) to (f) raw EOT signals at steady temperature region. Each color represents a different set temperature.

However, our results suggested that averaging should be done over the entire NHA site to get repeatable trends. This observation is also consistent with the PMT sensor results with less
noisy readings where the intensity measurements were done by averaging the entire view field. In Figure 3.49 and Figure 3.50, (a) to (c) indicate that averaging only a portion of the NHA site results in non-repeatability in the signal. 13x13 averaging gave a repeatable EOT signal trend when temperature was increased and decreased. This result indicates that, the collimated EOT light has a three dimensional characteristic. Temperature change not only affects the intensity magnitude but also the shape of the three dimensional Gaussian shape. For this reason, entire collimation should be taken into the account to get repeatable results.

Based on this results, 13x13 averaging was used in our experiments. To save on the memory space and read out speed, LabVIEW software was used to capture data only from the NHA sites instead of the entire image. At each NHA site, LabVIEW captured and averaged over a 13x13 pixel area and recorded to a spread sheet.

3.3.4. Temperature Sensitivity of NHA Sensors – Parametric Analysis

To study the dependency of NHA parameters on the temperature sensing characteristics, the following methodology was followed. The NHA matrix was centered in the CCD camera view. The temperature was increased from 28.00°C to 29.00°C in steps of 0.10°C (every 10 minutes). The EOT at all sites was monitored simultaneously using the CCD camera and recorded using LabVIEW software.
The mean value of the signal from each NHA site was taken over time at thermal equilibrium. The sensitivity was defined as the ratio of the change in the intensity to the light intensity value before the 0.10°C temperature change (base signal).

The EOT signal was measured during thermal transition and equilibrium phases. A raw transmission signal from a 10x10 NHA with pitch distance of 250nm is presented in Figure 3.52 (a). The EOT signal goes to a steady level as the thermal equilibrium was satisfied after each step change in controlled temperature. Figure 3.52 (b) shows the controlled temperature of the copper surface, measured by the thermistor. Green and red dots in Figure 3.52 (b) correspond to the steady state region and represent the region averaged for the calculations. Figure 3.52 (c) shows the room temperature over time where experiment was performed.
Figure 3.52: (a) Raw EOT signal from 10x10-250nm pitch NHA measured by the CCD camera. (b) Controlled temperature on the copper surface with step changes. (c) Ambient temperature.

Figure 3.53 shows the dependency of EOT magnitude on the NHA array size and pitch distance. Average transmission from each NHA site during constant temperature (28.20°C) was calculated. In accordance with the observations from Yang et al. [46], transmission increases with the increasing array size. In addition, as the pitch distance between the nanoholes increases, average transmission decreases.
Figure 3.53: Average EOT value measured in 12-bit CCD camera units at constant temperature (28.20°C) for 15 NHA sites.

For sensitivity calculations, normalized sensitivity was defined as the gain in the EOT signal due to a 0.10°C temperature change, normalized by the base signal value. This procedure was followed for all 15 NHA designs. Results of these calculations were summarized in Figure 3.54.

Figure 3.54 shows that the array size plays an important role in the temperature sensitivity. The highest transmission was measured from the p=250nm arrays; however, sensitivity was low compared to other pitch sizes, most likely due to the lack of sensing area in the array between the nanoholes. Between constant pitch distance arrays, the larger array (10x10) had higher sensitivity compared to the smaller arrays. In all array sizes, NHAs with pitch distances of 350nm and 400nm performed better in terms of normalized sensitivity with values
up to 8% change in the EOT signal due to a 0.10°C step change in the temperature. Beyond pitch values of 400nm, the sensitivity started to go down. This behavior can be attributed to the peak wavelength relation presented in Eq. (1.13).

Substituting dielectric constants for gold and water as the metal film and sensing layer respectively, the peak wavelength (maximum transmission at fixed temperature) can be estimated as a function of pitch distance (lattice constant, $a_0$ in Eq. (1.13)).

Figure 3.54: Normalized intensity gain in all 15 NHA designs due to a 0.10°C step change from 28.20°C to 28.30°C.

The result for this calculation is presented in Figure 3.55. By substituting our incident He-Ne laser wavelength, 632nm, as the peak wavelength to the Eq. (1.13), (pg. 27), lattice constant, $a_0$ (pitch distance), can be calculated as approximately 340nm. This suggests that, steep gradients in the transmission versus spectrum curve, due to the transmission maxima in this
region, result in higher response changes in the EOT signal in the neighborhood of this pitch value, leading to higher sensitivity. Our results show that this high sensitivity occurs between 350-400nm pitch distances.

![Graph showing transmission peak wavelength estimation as a function of NHA pitch size using Eq. (1.13).](image)

The baseline transmission intensity magnitude increased as the number of holes in an array increased, as expected. Decreasing pitch distance resulted in an increase in the intensity magnitude but a decrease in the sensitivity, perhaps due to the reduced metal film area. The highest sensitivity was achieved with 400nm pitch NHA sensors in 10x10 array and was measured to be 8% for a temperature step of 0.10 °C. These conditions, taking into account the incident wavelength, correspond approximately to a theoretically predicted peak transmission region with high gradients in transmission versus spectrum curve, resulting in higher sensitivity. This behavior was consistent in all array sizes. Results also showed that sensors as small as 0.65μm x 0.65μm area are sensitive to temperature changes, enabling measurements in small
volumes and at high spatial resolution. In combination with the theoretical studies, this result demonstrates that NHA parameters can be optimized in terms of temperature sensing, depending on the wavelength of the light source and the dielectric constant of the fluids used for the application. In addition, using a white light source, different wavelengths can be used to optimize temperature sensitivity as an extension of the conclusions derived in this section.

3.3.5. Calorimetric Reactions in Coflow Configuration

In this section, co-flow type reaction results are presented. A flowcell in gasket configuration having two inlet channels that merge into a single reaction channel, 450μm wide 100μm high, was clamped to the sensor chip, which was backed with a copper heat sink regulated at 25°C. The flowcell consisted of a PDMS gasket configuration and a glass cover as shown in Figure 2.11 (b). The fluid was in direct contact with the gold. The rows of sensors were aligned perpendicular to flow at two positions, 900 and 2200μm downstream of the inlet. Light from a LED light source with maximum power at 625nm traversed the flow cell to illuminate the sensors, and transmission through the sub-wavelength nanoholes was measured using a CCD camera. A CCD image showing the nanohole arrays and their locations in the channel is shown in Figure 3.56.

The light intensity at all sites was recorded for continuous flow of reactant A (5μL/min flow rate) in both inlets followed by coflow of reactant B in one inlet and reactant A in the other. The mean intensity change at each NHA sensor location was measured, where the mean was taken before and after coflow solution replaced A in one of the inlets.
Figure 3.56: CCD camera image of the reaction channel and the light transmission from the nanohole arrays. Channel walls are highlighted with red lines. The NHA sensors from 1 to 38 are shown with arrows.

Figure 3.57: Sketch showing three stages of the experiment. EOT was measured during 1. injection of the same reactant A, 2. coflow of A and B, 3. during the mass and thermal equilibrium state.
### 3.3.5.1. Ethanol-DI Water Dilution Tests

Three different experiments were performed with different concentrations of ethanol mixtures and pure DI water. The physical properties of water and ethanol reactants are listed in Table 3.5. In one inlet ethanol mixtures at different concentrations were introduced and water was the fluid in the other inlet. The details for the prepared mixtures were summarized in Table 3.6. Mole ratio, $X_{\text{ethanol}}$ was calculated as $n_{\text{ethanol}}/(n_{\text{ethanol}} + n_{\text{water}})$.

#### Table 3.5: Physical properties of water and ethanol.

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<th>Property</th>
<th>Ethanol</th>
<th>H$_2$O</th>
</tr>
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<tbody>
<tr>
<td>$\rho$ [kg/m$^3$]</td>
<td>789</td>
<td>998</td>
</tr>
<tr>
<td>$M$ [kg/mol]</td>
<td>46.07 \cdot 10^{-3}</td>
<td>18.02 \cdot 10^{-3}</td>
</tr>
<tr>
<td>Index of refraction $n$</td>
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<td>1.333</td>
</tr>
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</table>

#### Table 3.6: Parameters and calculated values for the prepared ethanol mixture in experiments. The volume and calculations are based on the volume for the prepared mixture, not the flowcell volume.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{ethanol}}$ [mL]</td>
<td>1</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>$V_{\text{water}}$ [mL]</td>
<td>0</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>$[C]_{\text{ethanol}}$ [mol/L]</td>
<td>17.13</td>
<td>13.70</td>
<td>3.425</td>
</tr>
<tr>
<td>$n_{\text{ethanol}}$ [mol]</td>
<td>0.017</td>
<td>0.014</td>
<td>3.425 \cdot 10^{-3}</td>
</tr>
<tr>
<td>$n_{\text{water}}$ [mol]</td>
<td>0</td>
<td>0.011</td>
<td>0.044</td>
</tr>
<tr>
<td>$X_{\text{ethanol}}$ [mol/mol]</td>
<td>1</td>
<td>0.553</td>
<td>0.072</td>
</tr>
<tr>
<td>$\Delta E_{\text{dilution}}$ [J/mol]</td>
<td>-392.02</td>
<td>-309.83</td>
<td>-24.16</td>
</tr>
</tbody>
</table>

In all experiments water was injected at a constant volumetric flow rate of 5μL/min flow rate in both inlets and liquid was switched to one of the mixtures listed in Table 3.6. For each experiment, energy release was calculated using the ethanol-water dilution chart presented in Figure 3.58. This plot was created using the data by Boyle et al. [87].
Using Figure 3.58, enthalpy of dilution per mole of ethanol was calculated for three mixtures used in the experiment. This calculation was done by integrating from the initial value of the mole fraction of the ethanol mixture to the pure water concentration level. Numerical trapezoidal integration was done in MATLAB. For example, for the pure ethanol experiment was done by integration from $X_{\text{ethanol}} = 1$ to $X_{\text{ethanol}} = 0$. Integration over this range resulted in a value of -392.02 J/moles. For the second experiment, integration from $X_{\text{ethanol}} = 0.553$ to $X_{\text{ethanol}} = 0$ gave a value of -309.83 J/moles. For the third experiment, $X_{\text{ethanol}} = 0.072$ to $X_{\text{ethanol}} = 0$ range gave a value of -24.16 J/moles. These results were also summarized in Table 3.6 for all three experiments.

![Figure 3.58: Enthalpy of dilution of ethanol-water mixtures.](image-url)
Intensity changes at the NHA sensors at two different z locations, $z_1 = 900\mu m$ and $z_2 = 2200\mu m$ along the flow direction were recorded as explained in the beginning of the section. Figure 3.59 presents the control volumes at $z_1$ and $z_2$ locations. This diffusion boundary can be identified using the EOT signal changes along the channel width, x-direction. For the first control volume at $z_1$, the EOT signal started to rise at the vicinity of sensor number 5 and this peak goes back to the concentration level in the vicinity of sensor 9. For the second control volume at $z_2$, the EOT signal started to rise at the vicinity of sensor number 4 and this peak goes back to the concentration level in the vicinity of sensor 10 (Figure 3.63 and Figure 3.64). This behavior is consistent with laminar diffusion interface. Since the distance between each NHA site was known (30µm), the inter-diffusion width at $z_1$ and $z_2$ can be estimated. At $z_1$, this value was 120µm and 180µm. The footprint of an NHA sensor used in this experiment was 3.3x3.3µm and the flowcell thickness 100µm. Using these values and Eq. (3.41), the total volume for the
first control volume was calculated as $V_{z1} = 120\mu m \times 3.3\mu m \times 100\mu m = 39.6\text{picoL}$ and for the second control volume at $z_2$ was calculates as $V_{z2} = 180\mu m \times 3.3\mu m \times 100\mu m = 59.4\text{picoL}$. Using the calculated volumes and dilution enthalpy per mole of ethanol, energy release for each experiment was calculated. Results of these calculations were reported in Table 3.7.

$$V_z = \text{Sensor width} \times \text{Channel thickness} \times \text{inter} - \text{diffusion region width}$$  \hspace{1cm} (3.41)

Table 3.7: Calculated values based on the control volume in the micro-channel.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1 (17.13M)</th>
<th>Experiment 2 (13.70M)</th>
<th>Experiment 3 (3.425M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Volume 1 [pL]</td>
<td>39.6</td>
<td>39.6</td>
<td>39.6</td>
</tr>
<tr>
<td>Control Volume 2 [pL]</td>
<td>59.4</td>
<td>59.4</td>
<td>59.4</td>
</tr>
<tr>
<td>$n_{z1}^{\text{ethanol}}$ [nanomoles]</td>
<td>0.68</td>
<td>0.54</td>
<td>0.14</td>
</tr>
<tr>
<td>$n_{z2}^{\text{ethanol}}$ [nanomoles]</td>
<td>1.02</td>
<td>0.81</td>
<td>0.20</td>
</tr>
<tr>
<td>$\Delta E_{z1}$ [nJ]</td>
<td>$-265.87$</td>
<td>$-168.10$</td>
<td>$-3.28$</td>
</tr>
<tr>
<td>$\Delta E_{z2}$ [nJ]</td>
<td>$-398.80$</td>
<td>$-252.15$</td>
<td>$-4.92$</td>
</tr>
</tbody>
</table>

Figure 3.60: Raw EOT signal during pure ethanol, water dilution plotted with respect to time at $z_1=900\mu m$. An average intensity was calculated by averaging the raw signal over time indicated with green and red circles.
Figure 3.61: Raw EOT signal during pure ethanol, water dilution plotted with respect to time at $z_1=2200\mu$m. An average intensity was calculated by averaging the raw signal over time indicated with green and red circles.

Figure 3.60 and Figure 3.61 show the raw EOT data during the experiment monitored at $z_1$ and $z_2$ locations respectively. These figures are important because they present different transition times (thermal diffusion and mass diffusion). Note that each row had 19 independent NHA sensors and hence 1-19 correspond to $z_1$ and 20 to 38 correspond to the $z_2$ location. In this particular channel design, all of the sensors were in the flow channel. The water runs through both inlets can be observed between zero and thirteenth minute. At approximately thirteenth min., ethanol came in from the second inlet (i.e. lower half in Figure 3.56), and the shift in the EOT signal can be seen due to the change in the index of refraction (transition from water to ethanol). This transition took approximately three minutes and can be seen at the sensors 10, 19, 28, 29, 38 which are all away from the diffusion interface and close to the channel walls (sensor locations with respect to channel are illustrated in Figure 3.56). The fluid in the first inlet (i.e. upper half in Figure 3.56) was not changed during the experiment and no signal change was observed in the sensors covering this part of the channel. This behavior can be monitored in 1, 5,
20, and 24 which were all close to the channel wall (sensor locations with respect to channel are illustrated in Figure 3.56). The sensors in the vicinity of the laminar diffusion interface zone had different responses compared to the remaining sensors. First, the transition time required to reach a steady signal was approximately 20 minutes, longer than the transition occurring in the sides of the channel. This behavior is consistent with the fact that, thermal equilibrium is first dependent on the equilibrium in the mass diffusion and also takes longer to achieve due to the high number of parameters affecting thermal conditions including thermal diffusivity constant of the materials, and heat fluxes in all directions. Heat flow fluxes are present in all directions inside the control volume including the chip surface; however this is not the case for mass diffusion. The red and green dots illustrate the EOT signal averaging regions representing the steady signal EOT value before and after the co-flow. The signal was averaged over a three minute interval which corresponded to 250 data points.

Figure 3.62 represents these averaged values of EOT signal plotted as a function of channel width indicated with the sensors numbers. The black lines represent EOT signal during water flow in both inlets, circle markers indicating the first row, and triangles indicating the second row, respectively. The red lines represent the steady signal value after water/ethanol co-flow. Comparing the red and black lines with the similar marker shapes gives a clear picture of the region of the fluid change and laminar diffusion interface. Figure 3.62 also represents an intermediate step in our post-processing methodology. Intensity difference between two time periods can be calculated by simply subtracting red lines from the black lines with the same marker shape. The peaks in the center of the red curves in Figure 3.62 are related to the released energy.
Figure 3.62: Raw EOT signal at steady state during water flow in both inlets, $t_1$, indicated as black and Raw EOT signal during coflow steady state, $t_2$, red lines. Both $z$ locations were reported.

Figure 3.63 and Figure 3.64 show the result of the intensity differencing procedure mentioned above for mixtures with different ethanol concentrations, at $z_1$ and $z_2$, respectively. The EOT intensity difference before and after the injection was plotted as a function of sensor number, representing the channel width. No normalization or curve fitting was performed during the calculations. The parameters of the mixtures are summarized in Table 3.6.

Both in Figure 3.63 and Figure 3.64, the black, blue and red solid lines represent signal changes due to the co-flows of pure ethanol/water, 13.70M ethanol mixture/water, and 3.425M ethanol mixture/water, respectively. The dashed lines represent the results from the mass diffusion model, and their values were scaled according to the EOT intensity difference curve at each concentration.
It is also important to note the relative signal shift due to the transition from water to ethanol mixtures was dependent on the concentration of the mixture. The index of refraction change from 1.333 to 1.360, (water to ethanol) resulted in a 20 CCD units EOT change for the pure ethanol case outside the reaction zone (near wall regions). This value was 15 CCD units for the second mixture and 10 CCD units for the third mixture. The refractive index dependent shift was consistent at both z locations.

In accordance with the numerical simulation, the diffusion region in the center widens in channel direction (x-direction) further in downstream direction. At \( z_1 \), as shown in Figure 3.63, the peak was between sensor 5 and 9, at \( z_2 \) the peak widened to an area between sensors 4 and 10 in Figure 3.64.

**Figure 3.63**: Intensity difference across channel width (x-direction) at \( z_1=900\mu m \) for three different concentrations of ethanol-water mixture. Dashed lines represent scaled diffusion only model.
Figure 3.64: Intensity difference across channel width (x-direction) at $z_2=2200\mu m$ for three different concentrations of ethanol-water mixture. Dashed lines represent scaled diffusion only model.

To check the dependency of the EOT signal rise in the diffusion region, the area between the EOT intensity curve (solid line) and the numerical mass diffusion curve was calculated as presented in Eq. (3.42). This peak region is related to the released energy. The integration was done in the control volumes illustrated in Figure 3.59. Control volume calculations were based on the experimental values (width of diffusion region). Integration was done numerically with MATLAB using trapezoidal method.

$$\Delta I_{EOT} = \int_{x_1}^{x_2} [\Delta I(x) - \text{Curve}_{\text{MassDiff}}(x)] \, dx \quad (3.42)$$

In Eq. (3.42), $x_1$ and $x_2$ represent the diffusion mixing boundaries (Sensors 5 to 9 for the first row and 4 to 10 for the second row), $\Delta I(x)$ represents the EOT intensity difference curve (solid lines with circle markers in Figure 3.63 and Figure 3.64), $\text{Curve}_{\text{MassDiff}}(x)$ represents the
curve obtained from the numerical mass diffusion model (dashed lines in Figure 3.63 and Figure 3.64). The results of this integration and their ratios are reported in the third column in Table 3.8. The integrated areas were then compared with the theoretically calculated energy release due to enthalpy of dilution and were reported in Table 3.8. Comparison was done by comparing the ratios of the theoretical energy release between experiment 1 and 2 (17.13M and 13.70M co-flow experiments), and ratios of the integrated areas in the experimental EOT intensity difference curves. The results are listed Table 3.8.

Results in Table 3.8 showed that the increase in the diffusion region was proportional to the theoretical energy release within than a 5% error. The first row compares the ratios of theoretical and experimental values at $z_1$ location between two different concentrations (pure ethanol and 13.70M ethanol-water mixture). The second row tabulates same comparison at $z_2$ location. The third row compares theoretical and experimental ratios between $z_1$ and $z_2$ during the pure ethanol/water test while the fourth row tabulates similar data for 13.70M ethanol-water mixture test. These numbers are representative of the energy release during the tests, and taking ratios allows comparing measured energy release to the theoretically calculated values. Proportionality in theoretical and experimental calculations was consistent when comparing the energy release between high and lower concentration experiments (experiment 1 and 2). The same consistency was observed when comparing the energy release between two rows within the same experiments ($z_1$ and $z_2$). The energy release was higher at the downstream ($z_2$ location) compared to the first row. This behavior was due to the wider diffusion region along the downstream.

Table 3.8 shows that the peak in the EOT signal is due to the energy release due to the mixing in the laminar diffusion region. The area under peak is directly related to the amount of
the energy release and this behavior is in accordance with the theoretically calculated values for ethanol/water binary mixtures. Using a reference measurement location inside the reaction channel, energy releases at different locations can be estimated within 5% by comparing EOT peak curves to the reference measurement.

Table 3.8: Results of the theoretical and experimental calculations. Superscript exp1 represents 17.13M ethanol mixture/water coflow and superscript exp2 represents 13.70M ethanol mixture/water coflow.

<table>
<thead>
<tr>
<th></th>
<th>Theoretical (based on Figure 3.58) [n/n]</th>
<th>Experimental (based on Figure 3.63 and Figure 3.64) [ΔI_EOT/ΔI_{EOT}]</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta E_{z1}^{exp1}$/$\Delta E_{z2}^{exp2}$</td>
<td>$-265.87/-168.10 = 1.58$</td>
<td>$57.85/38.50 = 1.50$</td>
<td>4.99</td>
</tr>
<tr>
<td>$\Delta E_{z2}^{exp1}$/$\Delta E_{z2}^{exp2}$</td>
<td>$-398.80/-252.15 = 1.58$</td>
<td>$84.23/55.60 = 1.52$</td>
<td>3.92</td>
</tr>
<tr>
<td>$\Delta E_{z2}^{exp1}$/$\Delta E_{z1}^{exp1}$</td>
<td>$-398.80/-265.87 = 1.50$</td>
<td>$84.23/57.85 = 1.46$</td>
<td>2.67</td>
</tr>
<tr>
<td>$\Delta E_{z2}^{exp2}$/$\Delta E_{z1}^{exp2}$</td>
<td>$-252.15/-168.10 = 1.50$</td>
<td>$55.60/38.50 = 1.44$</td>
<td>4</td>
</tr>
</tbody>
</table>

3.3.5.2. EDTA – CaCl$_2$ Coflow Tests

Using the similar experimental setup and parameters, co-flow reaction of EDTA and CaCl$_2$ was monitored. The chemicals used were taken from the Test Kit of the GE-MicroCal’s VP-ITC instrument. The flowcell and tubing were first was filled with 10mM MES buffer. Next, both syringes were filled 0.1mM EDTA (in 10mM MES buffer) and injected continuously at 5 $\mu$L/min flow rate. The reaction of EDTA and CaCl$_2$ is an exothermic reaction and the enthalpy of formation for this reaction is -17.61 kJ/mole. The EOT signal was monitored and next 1.0mM
CaCl$_2$ (in 10mM MES buffer) was introduced from the first inlet. In the second repeat of the experiment, the CaCl$_2$ inlet was switched (second inlet was used instead of the first inlet). A steady EOT signal was monitored during the co-flow of EDTA-CaCl$_2$ and intensity differencing outlined in detail in the previous section was applied to the signal. No normalization was applied to the raw data.

![Graph](image)

**Figure 3.65: Intensity difference across channel width (x-direction) at $z_1=900\mu m$ $z_2=2200\mu m$ for coflow reaction of 0.1mM EDTA and CaCl$_2$ solutions in MES buffer.**

Figure 3.65 shows the intensity difference before and after the co-flow of EDTA and CaCl$_2$. The black and red lines in the figure represent the measurements at the first row at $z_1=900\mu m$ and second row at $z_2=2200\mu m$. Since the concentration of the MES buffer solution is relatively low, overall intensity change due to index of refraction is in the noise levels. Based on the results in the previous section of ethanol/water mixture runs, it can be concluded that the peak observed in the second row during both experiments was due to the heat release. Based on the peak in the vicinity of sensor 10, it can be concluded that heat release was captured only at the second row, $z_2$ location.
To test the consistency of results, we repeated the same experiment and switched the CaCl$_2$ inlet. This resulted in a change in the peak location. Similar to the first experiment, heat release was not captured at the first row but a peak with the same magnitude was observed at $z_2$ location. This observation was also consistent with the previous water/ethanol runs since the results show that energy release was higher in the downstream due to wider mixing region.

Figure 3.66: Repeat of the experiment. Intensity difference across channel width (x-direction) at $z_1=900\mu$m $z_2=2200\mu$m for coflow reaction of 0.1mM EDTA and CaCl$_2$ solutions in MES buffer.

The concentrations used in this test were lower compared to the ethanol/water tests but a visible peak, (close to the signal noise level) in the mixing region was captured at the second row locations in both runs. This behavior can be due to the increase in the width of the laminar diffusion region which leads to higher energy release and the energy released in $z_1$ location is small.
4. CONCLUSIONS

Extraordinary optical transmission (EOT) signal was used as a temperature sensor to monitor temperature changes during a chemical reaction in microfluidic environments. A reactant delivery system with predictable diffusion characteristics and an optical interference free apparatus was designed for temperature induced EOT intensity measurements.

A four-port PDMS microfluidic system was designed and fabricated using micro-mold printing process. Salt dilution tests were performed in an 8.8μL reaction chamber. A series of saturated salt solution was injected to a chamber initially filled with DI water. Using a He-Ne (632nm) laser and a photo-multiplier tube (PMT), the transient response due to energy release and concentration change in the EOT signal was monitored during and after reactions. The concentration level inside the chamber increased and reached a constant level after several injections. This behavior was measured using the EOT signal once the mixing was complete and steady signal was observed. This increase in the signal was in agreement within 4.40% with the analytical model representing the concentration increase inside the reaction chamber. A complete numerical model of the system was built used to understand the transient response characteristics of the EOT signal in the case of a step temperature change. Numerical and experimental results showed that EOT response time is faster than the small thermistor we used in our tests (0.450mm head size).

In order to achieve controllable and predictable mixing within the microchannel, a different microfluidic approach was used. This approach helped us to distinguish the regions affected by the energy release using EOT signals. For this purpose, T-Sensor approach was employed where two streams merge into a single channel and mix due to laminar diffusion.
interface. The channel dimensions were reduced to 100\(\mu\text{m}\) in thickness, 500\(\mu\text{m}\) in width and 5000\(\mu\text{m}\) in length reducing the volume to 250nL (from 8.8\(\mu\text{L}\)).

An analytical methodology based on the general energy equation was derived to relate temperature readings at discrete locations on the chip surface and to the enthalpy of formation during a reaction. A 3-D numerical model was built simulating co-flow of an acid-base reaction with constant enthalpy of formation (NaOH and HCl). Simulations results were used to validate the analytical methodology. Using the simulation results, a sensor spacing analysis was performed by repeating calculations by taking temperature and concentration readings at discrete points spaced equally, similar to a Nanohole Array Sensor (NHA) configuration. This analysis was important because, a small sensor spacing value can cause interference between surface plasmons affecting the EOT signal. An optimum sensors spacing value is also important for nano-fabrication purposes. Simulation and analytical results showed that using surface measurements from 30\(\mu\text{m}\) spaced locations (sensors) can estimate the enthalpy of reaction within the 9\% of the reference value. This value reduced to 4\% for 20\(\mu\text{m}\) spaced intervals (distance between NHA sensors). 30\(\mu\text{m}\) sensor spacing value was used as a parameter for the chip fabrication.

EOT is sensitive to the changes within the thickness of a \(\lambda/4\) above the chip (flow) surface. \(\lambda\) is the wavelength of the incident light, and in our studies this micro-sensing region corresponds to a value between 100-300nm above the chip surface where NHA sensors are present. A thermal boundary analysis was performed inside the micro-channel to investigate if surface based measurements represented the temperature and concentration changes in the bulk of the fluid (entire channel). For our configuration, and simulation parameters, surface based temperature measurements taken from the micro-sensing region represented 98.58\% of the
average temperature (in the direction of channel thickness) at that sensor location. This result suggests that surface based measurement technologies, combined with microfluidics can accurately represent average properties of the liquid at the sensors location.

A parametric study was performed to investigate the temperature sensitivity of NHA sensors based on the array parameters. 15 different designs were milled in a formation of 3x5 matrix. Each row has a different array size (3x3, 5x5 and 10x10) and each column has a different pitch size varying from 250nm to 450nm in 50nm increments. The hole diameter was fixed at 150nm. The overall size of a single NHA array ranged from 0.65μm×0.65μm to 4.20μm×4.20μm. EOT signal was monitored simultaneously during a step temperature change and signal change normalized by the base signal value was used as a measure of temperature sensitivity. The highest sensitivity was achieved with 350nm and 400nm pitch sensors and a 10x10 array. These conditions correspond to a predicted peak wavelength region with high transmission gradients, due to the transmission maxima, causing higher sensitivity. The baseline transmission intensity magnitude increased as the number of holes in an array increased, as expected. Results also showed that sensors as small as 0.65μm×0.65μm area are sensitive to temperature changes, enabling measurements in small volumes and at high spatial resolution.

A NHA chip was fabricated based on the findings in the numerical simulation (sensor spacing) and parametric study (pitch distance and array size). Due to the nano-fabrication time, two rows of sensors were aligned perpendicular to the flow at two positions, \( z_1=900\mu\text{m} \) and \( z_2=2200\mu\text{m} \). Each row consisted of 19 NHA sites, 30μm apart from each other across the channel width. Each row covered the flow channel width entirely.
An interference-free optical configuration was developed for localized temperature measurements in microfluidic flow channels using NHA sensors. It was found that, a coherent light source creates interference patterns due to the reflections between the gold surface and top surface of the channel wall (glass or PDMS) and these interference patterns were sensitive to temperature and pressure. A series of iterative tests were performed with different optical components and flowcell assembly configurations to identify this problem and come up with a solution. This interference pattern was avoided by using an incoherent light source. The deformation on the upper wall of the PDMS channel created Newton’s rings type structures even when using an incoherent light source. Using a gasket type design and using glass cover as the upper wall of the flow channel solved this interference problem.

A NHA site covered approximately 11x11 pixel area in the CCD camera with a 10x lens. A study was performed to understand the importance of pixel averaging region. The temperature was changed in steps of 2°C from 28°C to 36°C and back to 28°C and images were captured at each steady state. Next centers of each NHA site was found using a script and average EOT signal was calculated using different averaging sizes of 3x3, 7x7 and 13x13. Only 13x13 averaging resulted in repeatable change in the EOT signal due to temperature increase and decrease. This result demonstrated that EOT collimation at the back side of the chip had a three dimensional Gaussian profile and changed in all dimensions due to temperature. 13x13 averaging size was used for all experiments.

Using an interference free optical configuration with LED light source, a polarizer, a condenser and a flowcell with a glass cover and gasket design, EOT vs. temperature plots were created for DI water and ethanol. The change in EOT with respect to temperature was repeatable and linear. It was found that EOT signal gain due to temperature change (28 to 36°C in steps of
2°C) was 3.810 times higher than the DI water signal gain. This behavior was explained by the difference in the volumetric expansion coefficient, which is a factor in the change of dielectric constant due to temperature changes. This trend was also in agreement with the analytical relations.

Using the same optical configuration and flowcell assembly with PDMS gasket and glass, co-flow reactions of ethanol mixtures/DI water and CaCl₂ and EDTA compounds were investigated. Three different concentrations of ethanol solutions were diluted with water and reaction was monitored using EOT signals. The intensity change was calculated before and after the introduction of the second reactant. A shift in the EOT signal due to index of refraction change was observed on the side of the channel where liquid transition from water to ethanol occurred. This shift was dependent on the concentration of the ethanol mixture. In the center of the channel, where the diffusion mixing occurred, a higher EOT signal was monitored caused by the energy release in the reaction zone. The magnitude of the peak was dependent on the concentration of the mixture. This conclusion was validated as follows. First, enthalpy of dilution was calculated for each mixture. Next, the area between the experimental EOT intensity difference curve and the scaled numerical mass diffusion model curve was calculated by integration. Finally, the ratios of theoretical and experimental values were compared. The results were in agreement with an error less than 5%. Similar co-flow experiment was performed with a Test Kit by MicroCal. The compounds were 0.1mM EDTA and 1mM CaCl₂, both in 10mM MES buffer. A peak due to heat release was observed in the downstream location for this reaction. This reaction demonstrated the sensitivity limits of this technology.
5. RECOMMENDATIONS and FUTURE WORK

Co-flow reactants used in the experiments can be extended to biological compounds, so that the energy release in different reactions related to life sciences can be observed and compared.

The metal film layer on the chip surface can be coated with an optically and biologically compatible material (PMMA, PDMS) to eliminate the effect of concentration in the sensing region (100-300nm normal to the flow surface where EOT sensing is effective) and isolate the temperature component of the signal. The effect of depositing this layer needs to be investigated. Microchannel reactor designs completely isolating concentration effects (such as PDMS or polycarbonate posts corresponding to NHA sites) can be a promising study.

Further investigation on the optical system can be performed to improve the temperature sensitivity of the experimental setup with the LED light source. A high powered tungsten white light source with a monochromator can make it possible to interrogate for the peak wavelength and adjust the sensitivity or dynamic range accordingly.

Following the previous suggestion, using the setup with the monochromator, transmission as a function of the wavelength at different set temperature points can be investigated. This could be useful for the calorimetric research as well as the affinity sensor since this can give a complete picture of the transmission behavior.

The EOT signal is convolved with temperature and concentration effects. This behavior is described in detail in Appendix A. A detailed study to apply a proper deconvolution technique in order to separate the temperature and concentration contribution on the EOT signal is important to further perform quantitative calculations.
A 3-D numerical simulation modeling the mass and thermal transient behavior in T-Sensor during the time between when two streams meet and the steady state is useful to understand the system characteristics. The same model can be used to assess the performance of a small plug and its interaction with the buffer liquid before it reaches the flowcell channel. The model can be extended to include the adsorption effects between the reactants and flowcell channel walls.

Developing a calibration methodology, where the known set point value for calibration (temperature or known amount of energy release) is applied inside the flowchannel or close to the NHAs. This approach can minimize the uncertainty between the set and calibrated quantities and also reduce the overall calibration time.
REFERENCES


47. Wang, W., et al., *Tuning the extraordinary transmission in a metallic/dielectric CDC hole array by changing the temperature*. Optics Express, 2010. 18(15).


APPENDIX A

EOT is affected by change in concentration change as well as temperature. A numerical estimation was done to understand how temperature and concentration was convolved in EOT signal. This numerical estimation was compared with the experimental results to prove the validity of the convolution approach.

![Graph of EOT vs Temperature for different Concentrations](image1)

**Figure A-1:** (a) A calibration plot (numerical) of EOT values for different concentration values at different temperatures. Mass diffusion (b) and temperature (c) profiles across a cross-section of a channel of a 6.24M salt solution in co-flow with pure water (numerical).

Figure A-1 (a) shows a numerical estimation of a calibration data for different concentration levels of salt-water solution (pure water to saturated salt solution). This calibration information can be produced experimentally as follows: i) introduce reactant A at a known
concentration level, ii) increase the temperature using the temperature controller and monitor the EOT signal, iii) repeat this step with a different concentration level. Profiles presented in Figure A-1 (b) and (c) are the signal values to be extracted from the EOT signal during a co-flow reaction. To understand the properties of the convoluted signal, values in Figure A-1 (b) and (c) are substituted into Figure A-1 (a) and an EOT value was calculated across the channel width (At x=400µm, T=25.15°C and C_A=6.24M corresponding to an EOT of 540 in Figure A-1 (a)). Repeating this step across the channel width (x-direction) resulted in the profile shown in Figure A-2 (a).

![Figure A-2](image_url)

**Figure A-2:** (a) EOT curve (numerical) generated by using Figure A-1 (a), (b) and (c). (b) Change in EOT intensity across the channel width, experimentally measured during a co-flow reaction of saturated salt solution and pure water at two different locations in the flow direction.

Figure A-2 (b) shows experimentally monitored EOT change across channel width during a co-flow reaction of saturated salt solution and pure water, plotted in two different locations in flow direction. The procedure and results of the coflow experiments were explained in Section 3.3.5 in detail. The EOT signal was monitored by two rows of sensors (19 in each row) in linearly distributed across the channel width. Each circle and triangle represents a discrete
sensor. The first two sensors on the left were left outside the flow channel for reference. The pulse in the center region is due to the heat release from the laminar diffusion mixing.

There are available deconvolution techniques such mutlivariate curve resolution alternating least squares (MCR-ALS), which was outlined by several researchers [88-91]. However, the method requires strict initial guesses for the curves to be deconvoluted and this is difficult to estimate for this application. This deconvolution technique also requires a 2-D correlation data set where the signal to be deconvoluted is mapped out in all variables involved. For this case, variables are concentration of each species (A, B, and AB or other reactants, products), and different temperature points within the temperature range of the reaction. This type of test requires quite long experimental time and high repeatability.