TRANSISTOR-BASED BIOSENSING:
EXPANDING THE FUNCTIONALITY OF FIELD EFFECT TRANSISTORS

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ABSTRACT

Since the invention of Polymerase Chain Reaction (PCR)-based amplification of nucleic acids by Kary Mullis in 1983(1), researchers have spent significant efforts to improve the sensitivity and selectivity of PCR assay and have dramatically enhanced its application(2). PCR is now an integral tool of modern biotechnology processes and biological identification. Due to the growing demands for on-site, rapid diagnosis, attention has been paid in realizing portable, fast, and low cost PCR machines. Through expanding the uses of the field effect transistor platform to include a novel design of heating/cooling, ultra-localized cell lysis, and electrical detection of nucleic acid amplification using an on-chip electrode, this thesis aims to enable the next generation of portable biosensors for primary care and on-site diagnostics.

Chapter 2 presents an overview of the current state-of-the-art of electrical biosensors. In keeping with the goal of complete, integrated systems, methods for both sample preparation and detection were evaluated. Electrical sample preparation methods provide a unique opportunity to utilize electrical fields for cell lysis, concentration, and sample flow without the need for additional moving parts or reagents. Electrical detection methods offer a means of reducing reagents by eliminating the need for optical labels. Together, electrical sample preparation and detection will aid the development of portable, low-cost integrated biosensors.

Chapters 3 and 4 look at utilizing transistors as platform for sample preparation and manipulation through DNA denaturation and cell lysis. Through application of a 10MHz, AC field between the shorted source-drain and the back-gate of the chip, fringing electric fields located just above the transistor surface are generated. These fringing electric fields can be used for dielectric relaxation of water molecules to generate heat for DNA denaturation as discussed in chapter 3, or to generate a transmembrane potential across a cellular membrane for ultralocalized cell lysis as discussed in chapter 4. These methods expand the
functionality of the transistor platform by extending their uses into the realm of sample manipulation.

Since the development of ISFETs for biosensing applications, transistors have been used for detection of biological analytes. Chapter 5 extends this functionality to electrical detection of nucleic acid amplification with an on-chip quasi-reference electrode. This method eliminates the need for a bulky/difficult to fabricate reference electrode and enables parallel detection of a large array of individual nucleic acid amplification reaction volumes. This method promises to reduce cost of PCR assays by eliminating optical components as well as improve the portability of the equipment by localizing the detection element to a disposable chip.

As discussed in the future outlook presented in chapter 6, the silicon CMOS compatible technologies introduced can be made portable, rapidly heat and cool reaction volumes, and remain inexpensive so the ultimate vision of an array of PCR reaction chambers, where the target sample can be interrogated against an array of primers, can be realized.

This thesis has added three new functionalities to the transistor platform. 1) Rapid DNA denaturation 2) Localized single cell lysis and 3) Electrical detection of amplification by-products with an on-chip electrode.
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CHAPTER 1
INTRODUCTION

General Objective

With outbreaks like *Listeria monocytogenes* in ready-to-eat meats and 2011’s deadly enterohemorrhagic *E. coli* in Europe, food safety-related crises have garnered increasing amounts of attention from both the public and media in recent years. As consumer concern about keeping food safe continues to grow, so does the imperativeness of developing a method to quickly and accurately identify foodborne pathogens. Traditionally, this has been a time-consuming process requiring days to achieve final identification. Shortening the time frame required for detection can drastically reduce the direct financial impact from a product recall—and, more importantly, save lives. Nucleic acid-based methods are still considered the gold-standard for detection and identification of microorganisms and viruses due to their high specificity and selectivity as compared to antibody-based assays. Recent technological advances in microfluidics and micro/nanotechnology present new opportunities for development of small, sensitive, single-use, point-of-care “Lab-on-a-Chip” diagnostic devices capable of providing a rapid analysis of nucleic acid amplification. We propose to develop the concept of a “Lab-on-a-Transistor” for: (i) *cell capture and thermal lysing*, (ii) *ultra rapid techniques DNA denaturation for performing nucleic acid amplification* and (iii) *rapid, electrical detection of the amplified products on silicon transistors*. Although I will focus on food as a model system, our proposed approach can be used for detection of any disease or condition that requires nucleic acid based testing.
Specific Goals of the Thesis

Through this thesis, I aim to extend our lab’s extensive prior work of cellular concentration and detection using electrical methods to develop a “Lab-on-a-Transistor”. The following specific aims will guide us to the end objective:

Overview of Electrical Biochips – To better understand the breadth of options available in the world of electrical detection, a review of electrical detection biosensors will be covered. By covering electrical methods for sample preparation as well as detection, future options for completely integrated electrical systems will be introduced. Together, these methods will enable the next generation of portable biosensors. To aid that potential, later chapters will focus on expanding the library of functionality of the field effect transistor platform.

Sample Preparation: Localized Heating for Cell Lysis and DNA Denaturation – Using a FET platform developed from earlier work in the Bashir group, I will perform ultra-localized cell lysis and DNA denaturation above the surface of the field effect sensor using an applied AC voltage.(3; 4) These methods advance the capabilities of FETs and open up doors to a variety of applications including droplets for hybridization detection of low copy number DNA molecules, lysing of single cells, interrogation of ligand-receptor interactions, and rapid temperature cycling for amplification of DNA molecules.

Electrical Detection of LAMP By-Products – In further developing the FET platform, I will utilize foundry-level FETs for detection of nucleic acid amplification. The field effect transistor will be employed to electrically detect hydrogen ions generated as by-products of an amplification reaction. To extend beyond previously published material from Toumazou et al.(5), an on-chip, solid-state, quasi-reference electrode and a pH-insensitive REFET will enable electrical
detection of pH changes from loop mediated isothermal amplification. This method advances the potential for highly portable, massively parallel, electrical detection of nucleic acid amplification.

With this thesis, the transistor extends its functionality as an essential unit of electronic memory and microprocessors and goes from serving as a critical component of ‘Lab-on-a-Chip’ to the integral device in ‘Lab-on-a-transistor’. The transistor becomes a multifunctional tool capable of cell lysis, DNA denaturation and nucleic acid amplification by-product detection. This augments the use of silicon field effect transistors to new functionalities not yet described or pursued. Figure 1.1 schematically describes the specific aims and the workflow. Future work will focus on integrating the proposed system into a cartridge, which will be inserted into a portable device that performs PCR or LAMP followed by electrical detection.
Figure 1.1 Flow for proposed device: (a) shows the device cross-section. FETs located at the bottom of a reaction will can function as either heaters or detection elements for a biological reaction. (b) Compartmentalization of individual reactions through micropipetting or microfluidic well loading can be used to generate massive arrays. (c) After compartmentalization, a cell in solution can be lysed using the AC heating or electroporation technique. (d) Through AC heating or bulk heating of the array, PCR or LAMP can be carried out. (e) Through the use of an on-chip solid state electrode and a REFET, electrical detection of pH, pyrophosphates, or nucleic acids within each droplet is possible.
CHAPTER 2
LITERATURE REVIEW OF ELECTRICAL BIOSENSORS

Abstract

Electrical biochips utilize the power of microfabrication and electrical techniques for biological applications. Electric fields can be used to both manipulate and sense biological entities with several inherent advantages, including on-chip sample preparation, label-free detection, reduced cost and complexity, decreased sample volumes, increased portability, and large scale multiplexing. The advantages of fully integrated electrical biochip platforms are particularly attractive for point-of-care systems. This review summarizes these electrical lab-on-a-chip technologies and highlights opportunities to accelerate the transition from academic publications to commercial success.

This work has been submitted for publication as Reference [(6)].
1 Introduction

The field of diagnostics is a cornerstone of modern health care throughout both the developed and the developing world. It is becoming increasingly important to develop highly sensitive, specific, and rapid methods for detection of relevant biological entities. Such biosensor schemes are critical not only to garner vital patient information in a timely fashion, but also to accelerate the progress towards a better understanding of the complex biological pathways that govern disease. Electrical biosensors can offer a simple and inexpensive means to elucidate biological pathways, diagnose diseases, and save lives – particularly for point-of-care (POC) applications, where portability and cost are critical concerns. This review will provide an introduction to basic concepts behind POC electrical biosensing and present a critical overview of active research for emerging technologies to improve electrical sample preparation and detection techniques for the analysis of relevant biological entities, with focus on small molecules, nucleic acids, proteins, and cells.

Towards Decreasing Complexity in Biosensors

An ideal biosensor has the least amount of complexity while yielding the most desirable data. As illustrated in Figure 2.1, portable glucose sensors for diabetes patients represent one end of the spectrum of commercially available biosensors. Portable glucose sensors are typically quite cheap, yield quantitative results in minutes, and require almost no infrastructure, but are useful only for one specific application, the detection of glucose. At the other end, tools such as next generation sequencing machines or DNA microarrays yield an abundance of information for a variety of applications such as the sequence of an entire genome for disease pathogenesis. However, sequencing machines with such accuracy have high prices, take a few weeks to yield results, and require highly trained staff and advanced lab equipment. The dotted red circle in Figure 2.1 represents a significant void of commercially available devices, which deliver a high amount
of information per test with lower complexity. To fill this void, researchers have envisioned the development of point-of-care (POC) devices – cheap, portable devices capable of providing data quickly and accurately at the bedside of a patient without the need for specialized technical staff or expensive equipment. By decreasing complexity, POC devices aim to decrease the cost per test and increase the frequency of useful diagnostics performed across the world (7; 8).

**Figure 2.1 Classifications of Diagnostic Tests:** A tradeoff usually exists between increased complexity and information yielded per diagnostic test. As complexity of the test increases, possible locations for testing can be restricted to hospitals or only research facilities. Point-of-care sensors aim to fill the void in available tests by offering lower complexity tests that provide a large amount of useful information

*Basic Definitions and Concepts*

To start, an **electrical biochip** is any technology that uses either external applied electric fields to manipulate or sense biological molecules or the intrinsic electric
field of the biological molecules themselves to influence output characteristics on
a chip-based platform. The elimination of the need for off-chip sample
preparation or optical readout machinery can translate to important advantages for
POC sensors. This section will introduce basic concepts that are common to many
biosensors addressed in this review, including isoelectric point, Debye length, and
various electrode types. We will also cover important biosensor performance
metrics, such as resolution, signal to noise ratio, and sensitivity.

One of the key concerns for several electrical biosensors is the charge of
the target entity; this charge should be maximized in most cases to induce a
maximal signal change of the sensor. Once a biomolecule is placed into an ionic
solution, it will have an overall charge density on its surface that is highly
dependent on the pH of the surrounding solution, as shown in Figure 2.2A. The
*isoelectric point (pI)* of a molecule or surface is the pH of the solution at which a
biomolecule has a net zero charge. As the pH of the solution is decreased away
from the isoelectric point, the molecule will accrue more and more negative
charge; conversely, as the pH increases, the molecule becomes more positive. For
most sensor applications, it is desirable to maximize the charge of the molecule.

When a charged particle is placed into an ionic solution, the mobile ions in
the solution move to shield the charge, which can reduce the apparent charge seen
by a sensor and thus minimize sensor response. This forms the *double layer*, as
shown in Figure 2.2A, the charge at the surface of the molecule and the counter-
ions that surround the molecule. Ions that can be specifically adsorbed directly at
the surface make up the *Stern layer*, which is the layer closest to the charged
particle and is typically a few angstroms thick(9).
Outside the Stern layer is the diffuse layer, which contains a concentration of mobile ions that decays exponentially according to a Poisson-Boltzmann distribution, resulting in an exponential decay of the induced molecular electric field and the surrounding potential. The distance it takes for the induced electric

Figure 2.2 Electrode Schematics: (A) Polarizable Electrodes: With these electrodes, no Faradaic current flows and both the electrodes and charged molecules will be shielded by mobile ions in solution. (B) Non-Polarizable Electrodes: Faradaic current can flow, allowing ion exchange between the solution and the electrodes. This enables an electrophoretic force to be applied to a charged molecule. If an enzyme attaches to the molecule, the byproducts of the reaction can be used to quantify target molecule concentration. (C) Nanopore Sensor: A nanoscale sized gap separates two chambers, a cis and a trans chamber. Voltages applied to electrodes in these chambers cause ions to flow through the nanopore, inducing a measured ionic current that is modulated when DNA passes through the pore. (D) Potentiometric Sensor: A charged particle can be captured on the surface using an appropriate capture probe. The electric field lines generated by the particle will be partially shielded by ions in the fluid, reducing the induced charge on the surface of the device.
field from the molecule to decay to 1/e of its value at the beginning of the diffuse layer is called the Debye length, and is given by:

$$\kappa^{-1} = \frac{\sqrt{\varepsilon_r \varepsilon_o k_B T}}{2 N_A e^2 I}$$

where $I$ is the ionic strength of the electrolyte, $\varepsilon_r$ is the dielectric constant, $\varepsilon_o$ is the permittivity of free space, $k_B$ is the Boltzmann constant, $T$ is the temperature in Kelvins, $N_A$ is Avogadro’s number, and $q$ is the elementary charge. For a relevant physiological electrolyte, such as serum (~0.14 M), the corresponding Debye length is around 0.7 nm. Charge shielding for a potentiometric sensor is illustrated in Figure 2.2D, where a charged protein is tethered to a surface via a capture antibody. In this case, the charge of the molecule is mirrored by the sum of the charge of the screening counter-ions and the induced charge in the sensor.

Various types of electrodes are integral components of biosensors. An ideal polarizable electrode is an electrode which allows no transfer of electrons or ions to and from solution (no Faradaic current); an ideal non-polarizable electrode is an electrode where such transfer is possible. A polarizable electrode experiences similar screening effects as molecules (Figure 2.2A), and has the same Debye length in a given solution. In this case, even when voltages are applied by polarizable electrodes, a charged particle well outside a Debye length’s away from the electrodes will feel minimal electrostatic forces. However, because non-polarizable electrodes allow Faradaic current flow across the solution/electrode interface, ions will pass into the electrodes and electrophoretic forces can be applied to the molecule for manipulation (Figure 2.2B).

Several important criteria can be used to evaluate the efficacy of sensors. A fundamental parameter is the device’s resolution, or the smallest shift in target concentration the device is able to distinguish at a given initial target concentration. Device resolution can be quantified as $\alpha$/SNR, where $\alpha$ is a
confidence parameter usually chosen to be 3 or higher and SNR is the device’s **signal-to-noise ratio**. In a real world sample, where several non-target biomolecules are typically present along with the target, the device **signal** is generally considered to be the average raw response in output characteristics to a change in concentration of the target analyte. The **sensitivity** is defined to be the average difference in output from solution 1 to solution 2 divided by the difference in the two concentrations. **Sensor noise** is generally the sensor’s overall average response to anything in the system besides the target analyte, including fluctuations in solution potential, non-specific adsorption of molecules, and intrinsic device noise. This can be quantified by the average output response of the device to switching between two test solutions that contain the identical background, but with the same target analyte concentrations. One of the dominating contributing factors to device noise is the lack of perfect **selectivity** of the biological interface to the target analyte. Selectivity is defined as the ability of the device to distinguish the target analyte from a background of all other entities in the solution. A device’s **limit of detection (LOD)** is the lowest concentration the sensor can reliably detect. Most works do not adequately quantify device SNR or statistics of device response to really determine their LOD. Device **dynamic range** is the ratio of LOD to the highest detectable concentration. Dynamic range, LOD, and resolution are critical criteria for evaluating a biosensor.

**Components of a POC Electrical Biosensor**

A true POC device ideally inputs a raw sample from a patient and outputs useful information. To do this, several distinct components are necessary, as is shown in Figure 2.3. A **sample loading** module is necessary to introduce the raw sample to the device. **Sample preparation**, where lab steps such as manual centrifuging, pipetting, and mixing are ported on-chip using filters, microfluidic sorting, and concentration techniques, is a critical part of any POC device to transform the raw sample into a format that can interact appropriately with sensor
components. The resulting fluid then interacts with the biological interface of the POC device, which is the membrane or solution that is sensitive to the specific target analyte of interest. This interface creates a signal, which can be light, mass changes, surface properties changes, or electrical signals upon interaction with the target analyte. This signal is then converted into an electrical output by the signal transduction component. Finally, for many applications with high volumes of data, a signal processing or bioinformatics step is needed to filter out the most relevant information. Electrical biochips offer several inherently attractive advantages to improve the sample preparation and signal transduction components of a POC device. Several electrical techniques can be used on-chip for sample preparation, including cell lysing, concentration, and flowing/mixing techniques. The use of electrical sensors can minimize or completely eliminate the need for a signal transduction component of POC device, since the sensor itself produces an electrical signal that does not need to be converted. The remainder of this review will focus on electrical techniques for sample preparation and signal transduction.

Figure 2.3 Main Components of a POC Device: Electrical biosensors offer inherently attractive advantages, particularly for the sample preparation and signal transduction modules of POC devices.
2 Electrical Methods for Sample Preparation

While electrically-based biological detection techniques have developed significantly in recent years, most of these efforts focus on detection and not on the equally integral initial sample preparation steps.(12-14) Current methods for sample preparation in commercial systems are typically heavily reliant on manual labor and cumbersome equipment. For instance, DNA extraction from a complex sample requires multiple pipetting steps and subsequent centrifugations in order to adequately prepare a sample for analysis. (15) Each of these manual steps increases result variability, sample exposure to contamination, required technician training, and technician exposure to potentially dangerous pathogens. In the point-of-care setting, space, time, and equipment are limited and as a result, steps such as pipetting and centrifugation are not feasible. Without integrated on-chip sample preparation modules, portable, cost-efficient, fully automated lab-on-a-chip systems will see limited usage. Electrically-based sample preparation methods that utilize on-chip electrodes provide a unique opportunity to integrate cell lysis, concentration, and flowing/mixing with simple structures already present on the chip.

Cell Lysis

Cell lysis releases cellular components for future analysis, a necessary step in sample preparation techniques. Cell lysis is typically achieved chemically(9), enzymatically(10), thermally(12), and mechanically (16). However, electrical methods for cell lysis such as electroporation offer a few advantages. They can alleviate the need for lysis reagents that are incompatible with downstream processes, and can also decrease complexity by eliminating the need for added temperature control(17; 18). Without inhibitory chemicals or high temperatures, electroporation causes the release of cellular contents, allowing for molecular analysis of released contents with minimal damage. Electroporation utilizes either an AC or DC electric field to create a potential across the cell membrane. Pores
form in the cellular membrane once the transmembrane potential exceeds a threshold of ~0.2-1.0V.(19) These pores will either remain open (irreversible electroporation) or reseal (reversible electroporation). Factors that contribute to the variation in size and number of pores formed include the duration and field of applied electrical field, cell type, developmental stage, medium, and cellular dimensions. While robust, low-power electrical methods to extract cellular contents from single cells have yet to be commercialized, Jokilaakso et al., recently showed single cells lysis by positioning cells on top of a nanowire and applying an AC electric field from the nanowire to a backgate contact(18). Additionally, several reviews have been published that highlight recent trends in the electroporation field.(20; 21)

Concentration

An integrated biosensor often requires biomolecule concentration or sorting to separate target molecules from an undesirable background. In terms of electrical methods, cell contents can be manipulated using alternating current (AC) fields, direct current (DC) fields, or electrostatic methods. Specific techniques using electric fields for sample concentration include dielectrophoresis (22), electrophoresis (23), and others (24).
Pohl’s pioneering dielectrophoresis work demonstrating manipulation of polarizable particles in a non-uniform electric field in the 1950’s (13) has since
been adapted for use in biochips for filtration (25), concentration (22), capturing (26), and patterning cells (27). In DEP, a non-uniform electric field exerts a force on a particle in a direction dependent on the comparative complex permittivity of the particle and the medium. The complex permittivity is dependent on the dielectric constant ($\varepsilon$), the electrical conductivity ($\sigma$), and the frequency of the applied field ($\omega$). As depicted in Figure 2.4A and shown in the equation below, when the particle’s complex permittivity ($\varepsilon^*_p$) is greater than the surrounding medium ($\varepsilon^*_m$), the particle experiences positive DEP and is forced to the region of highest electric field such as the edge of an electrode. Negative DEP occurs when $\varepsilon^*_p < \varepsilon^*_m$ which results in the particle being forced to the region of lowest electric field, such as directly on top of the electrodes. One implication of the dependence on permittivity, conductivity, and frequency is that within a mixed sample, different cells may experience different forces, enabling cell sorting. This can be based on cell type (28), cell cycle phase (29), and viability (30). However, DEP is hindered in highly ionic solutions due to electrode screening and Debye length limitations, which has reduced its commercial applications.

\[
F_{DEP} = 2\pi \varepsilon_m r^3 \left\{ Re \left( \frac{\varepsilon^*_p - \varepsilon^*_m}{\varepsilon^*_p + 2\varepsilon^*_m} \right) \right\} |\nabla E_{rms}|^2, \varepsilon^* = \varepsilon + \frac{i\sigma}{\omega}
\]

Electrophoresis uses DC fields applied by Faradaic electrodes in place of AC fields to manipulate biological molecules. The traditional methods include gel electrophoresis and capillary electrophoresis, which have each been used for biomolecule separation and analysis for decades. For example, capillary electrophoresis systems such as Agilent’s Genetic Analyzer, have been instrumental to genome sequencing in the last 10 years (31). This method requires a desktop setup that typically takes 1-3 hours to run. In the last 20 years, researchers have pushed the boundaries to smaller, faster, less expensive microcapillary electrophoresis systems implemented on biochips for such applications as biomolecule separations (32), DNA separation (33), and protein
separation(34). These systems utilize microfabricated capillaries that require smaller sample volumes, shorter run times, and can have integrated detection capabilities. For example, capillary electrophoresis separations have been integrated with impedance, potentiometric, and amperometric detection modules(32; 35). However, it should be noted that DC electrophoresis often requires high voltages and causes electrolysis, which can lead to undesirable effects such as degradation of target molecules or surface functional layers (36).

For years, researchers have been able to perform biomolecule separations using electrostatic methods by allowing the biomolecules to interact and passively absorb to a surface of opposing charge. Developments in microfabrication in the past decade have utilized microfluidics in conjunction with microfabricated silica beads or pillars to allow miniaturized biomolecule separations on-chip(37; 38). By sequentially introducing the sample, wash buffer, and elution buffer, this method presents an extremely simple way to purify DNA from an initial sample using intrinsic electric charge and the isoelectric point of the DNA and the surrounding structures.

Flowing and Mixing

Flowing and mixing, integral requirements for sample preparation for biosensors, can be accomplished through the application of an AC or DC electric field to a solution. Electroosmosis (EO) and electrowetting-on-dielectric (EWOD) are methods utilizing this technique to induce movement of a fluid.

In DC EO, application of a field across a microfluidic channel causes the movement of ions in the electric double layer at the surface of the microfluidic channel (39). This movement drags the rest of the fluid along, resulting in net movement. On the other hand, AC EO typically applies an AC signal between two planar electrodes on a microfluidic channel’s surface. As shown in Figure 2.4B, the AC signal induces a double layer of counterions on the electrode
surface, with the portion of the electric field that is tangential to the electrode surface interacting with the electrical double layer to induce fluid movement in the system. Fluid movement takes place as counter rotating vortices in between the electrodes, allowing mixing of reagents. To induce flow, a traveling wave of AC signals down a channel can be used, or a DC offset which causes asymmetric charging of electrodes and a resultant net flow (40; 41). DC EO, like DC electrophoresis, involves high voltages that can cause electrolysis and pH variations, negatively impacting biomolecules. On the other hand, AC electroosmosis does not require high voltages, will not cause electrolysis, and has been shown to provide significantly higher flow rates of several hundred microns/second (42).

Finally, electrowetting-on-dielectric (EWOD) has garnered increasing interest in recent years as a means to implement large scale, digital microfluidics applications (43-45) by enabling sample movement, mixing, and separations. As depicted in Figure 2.4C, a solution is placed between two parallel plate electrodes coated with a dielectric. The top electrode serves as a ground, while the bottom surface includes multiple, individually addressable electrodes arranged in an array. A droplet is introduced to the parallel plate channel and a potential is applied between the top and bottom electrode. This applied potential changes the interface potential between the solution and dielectric and altering the droplet’s contact angle ($\theta_1$ vs. $\theta_2$). By switching on an adjacent electrode, a deformation in the edge of the droplet will occur due to the change in contact angle, in turn causing a pressure difference across the droplet and causing droplet movement. Arranging droplets in a parallel formation and programming switching between electrodes allows for multiple droplet movement and mixing. EWOD-based purifications of targeted biomolecules from a complex solution utilize systems that typically employ magnetic beads to tag a targeted analyte (46). The magnetic beads are then held in place while the sample droplet is moved, wash buffers are
brought in, and finally an elution buffer used to enable downstream sample analysis.

**Perspectives**

To date, few commercial lab-on-a-chip systems have demonstrated full integration of true sample preparation and on-chip detection. As described in the next section, great strides have been made towards development of new detection methods; however, without adequate sample preparation, lab-on-chip usage will be limited. A major limitation in utilizing electrical methods is the need for low ionic strength solutions. Without a method to sufficiently exchange solutions, Debye screening and decreased field strength at relevant distances from the electrodes will hinder electrical sensing and electrophoretic forces. Additionally, for biological systems, the cost and benefits of altering the ionic strength must be balanced. Low ionic strength solutions offer attractive benefits towards the aforementioned Debye length and electrophoretic forces; however, altering the salt concentration can lead to reduced cell viability, limited biomolecular binding affinity, and reduced enzymatic activity. However, companies that are able to balance these issues are benefiting from the usage of electrical components for sample preparation. For example, Advanced Liquid Logic, now owned by Illumina, has developed a system that utilizes electrowetting-on-dielectric to drive a digital droplet microfluidics platform. This technique has enabled a variety of applications including protein binding and activity analysis, qPCR, and sequencing. In doing so, Advanced Liquid Logic was able to utilize structures directly incorporated with the chip and the intrinsic charge of their solution to add functionality to the biochip platform. These sorts of combined, integrated systems can simplify system design and bring true lab-on-a-chip closer to reality.

3 **Overview of Electrical Biosensors**
Electrical biosensors can be divided into categories based on how the analyte interacts with another entity to induce a response that can be transduced into an electrical signal. These perturbations include potential changes, impedance changes, and current changes. Figure 2.2 can be used as a general overview for these different sensing methods. Figure 2.2B demonstrates how a target analyte can react with another entity, such as an enzyme, in solution to produce electrons and thus perturb the measured current between the two electrodes. This change in current can be correlated to the concentration of the target analyte, an example of an amperometric sensor. Even if such a chemical reaction were not used, the field lines between the two electrodes can be perturbed by the presence of a target entity, especially if the entity is quite large with high impedance, such as a cell. This is an example of an impedance biosensor, where the continuously measured impedance of the solution is modulated by the presence of a target entity between the two electrodes. This category includes Coulter counters and their nanoscale versions, nanopores (Figure 2.2C). Finally, the affinity-based biosensor shown in Figure 2.2D is an example of a potentiometric biosensor, where the intrinsic charge of the target analyte induces electric fields that interact with the sensor, modulating the potential on the surface.

This section will cover the prominent electrical biosensor device mechanisms – impedance, potentiometric, and amperometric sensors. A table presenting some results from several sensing technologies is shown in Table 1. Each subsection will provide an overview of the most prominent published literature, followed by a perspectives portion, which will focus on the commercialization possibility of each technology and its future potential. This will include an evaluation of the technology utilizing five critical metrics (low-medium-high): (i) ease of device fabrication, (ii) amenability for multiplexed detection, (iii) complexity of input solution, (iv) sensitivity and (v) commercialization status.
<table>
<thead>
<tr>
<th>Entity</th>
<th>Technology</th>
<th>Sample Preparation</th>
<th>Linear Range</th>
<th>Detection Limit</th>
<th>Most Complex Starting Buffer (Debye length if applicable)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ Cells</td>
<td>Solution Impedance</td>
<td>Centrifugation and off-chip dilution to buffer</td>
<td>0-3000 cells/μL</td>
<td>20 cells/μL</td>
<td>Human Blood</td>
<td>(47)</td>
</tr>
<tr>
<td>cDNA from RNA</td>
<td>Standard DNA Microarray</td>
<td>Off-platform purification</td>
<td>0.2 nM- μM range</td>
<td>0.2 nM</td>
<td>Human Blood</td>
<td>(48)</td>
</tr>
<tr>
<td>PCR Product</td>
<td>Affinity Impedance</td>
<td>DNA precipitation and purification to buffer</td>
<td>1-100 pM</td>
<td>1 pM</td>
<td>Cell Lysate in PCR Solution (1 nm)</td>
<td>(49)</td>
</tr>
<tr>
<td>20 bp DNA</td>
<td>Potentiometric NWFET</td>
<td>Spiked Buffer</td>
<td>10 pM-100 nM</td>
<td>10 pM</td>
<td>1X SSC Buffer (0.7 nm)</td>
<td>(10)</td>
</tr>
<tr>
<td>35 bp DNA</td>
<td>Potentiometric NWFET</td>
<td>Spiked Buffer</td>
<td>30-100 fM</td>
<td>10 fM</td>
<td>1 μM phosphate buffer (&gt;10nm)</td>
<td>(50)</td>
</tr>
<tr>
<td>PSA</td>
<td>Standard ELISA</td>
<td>None Required</td>
<td>1-45 ng/mL</td>
<td>1 ng/mL</td>
<td>Human Blood Serum</td>
<td>(51)</td>
</tr>
<tr>
<td>PSA</td>
<td>Affinity Impedance</td>
<td>Dilution with Buffer</td>
<td>30-120 ng/mL</td>
<td>1.1 ng/mL</td>
<td>Human Serum</td>
<td>(52)</td>
</tr>
<tr>
<td>PSA</td>
<td>Potentiometric FET</td>
<td>Microfluidic Purification Chip to buffer</td>
<td>1.5-5.5 ng/mL</td>
<td>1.5 ng/mL</td>
<td>Human Blood (0.7 nm)</td>
<td>(53)</td>
</tr>
<tr>
<td>PSA</td>
<td>Potentiometric FET</td>
<td>Centrifugation with Filter to buffer</td>
<td>0.1 pg/mL-1 ng/mL</td>
<td>0.1 pg/mL</td>
<td>1 μM phosphate buffer (&gt;10 nm)</td>
<td>(54)</td>
</tr>
<tr>
<td>Glucose</td>
<td>Amperometric</td>
<td>Manual Dilution in Buffer</td>
<td>0.4μM-1.2mM</td>
<td>0.2μM</td>
<td>Human Blood Serum</td>
<td>(55)</td>
</tr>
<tr>
<td>Glucose</td>
<td>Amperometric</td>
<td>Spiked Buffer</td>
<td>100μM-10mM</td>
<td>5.8μM</td>
<td>1X PBS</td>
<td>(56)</td>
</tr>
<tr>
<td>Lactate</td>
<td>Amperometric</td>
<td>Spiked Buffer</td>
<td>14-325μM</td>
<td>4.1 ± 1.6μM</td>
<td>0.1M Sodium Phosphate Buffer</td>
<td>(57)</td>
</tr>
<tr>
<td>Lactate</td>
<td>Flexible Epidermal Amperometric</td>
<td>None Required</td>
<td>1mM – 20mM</td>
<td>1mM</td>
<td>Epidermal placement</td>
<td>(58)</td>
</tr>
</tbody>
</table>

**Table 2.1 Characteristics of Electrical Biosensors Using Various Technologies**

3.1 Impedance Based Biosensors

Impedance based schemes for biosensors measure the impedance between two electrodes, which can be Faradaic or non-Faradaic, in solution. This impedance is a complex quantity that can be used to approximate resistance,
capacitance, and inductance values. Without the presence of the target entities, the impedance is dominated by the intrinsic impedance of the surrounding solution. When a target entity interacts with the field lines between the two electrodes, the magnitude of the induced measured impedance change can be correlated to the concentration of the target entities. To measure this impedance, typically the current response to an applied AC voltage is measured and divided by the applied voltage to extract the impedance of the system. One of the primary constraints for impedance sensors is the requirement that the target entities significantly affect the impedance of the surrounding system, which is optimized for very large targets such as whole cells, for very small interrogation volumes, and for systems where the target entities are constrained to close proximity to the electrodes. This requirement limits the applications for which impedance based biosensors can be used; however, the simplicity and ease of fabrication of these techniques offer significantly attractive advantages for several applications.

**Microscale Impedance Sensors**

Microscale solution based impedance biosensors can be used to detect larger entities such as cells. These sensors can be used for cell counting applications, from detection of bacteria to monitoring a patient’s health status through a complete blood count (CBC). Devices can monitor the overall change in impedance of a solution containing a certain concentration of cells either as the cells are lysed or as they exhibit characteristic changes as part of normal metabolic activity. Alternatively, devices can count cells individually as they pass between the two measuring electrodes and induce changes in the measured impedance, utilizing the Coulter principle. Liu et al. demonstrated the electrical detection of the germination of spores of *Bacillus anthracis* using interdigitated electrodes(59). Cheng et al. demonstrated the use of impedance spectroscopy for the counting of CD4+ T cells from whole blood utilizing an antibody functionalized capture chamber for monitoring of HIV patients(47). This work
has since been used as the fundamental technology by Daktari Diagnostics for global health applications. Watkins et al. developed a microcytometer chip for the same target CD4+ cell counting applications using the Coulter counting principle (60). This chip utilized a differential counting scheme, where the count was measured using the difference in cell count between an input sample containing whole blood and an output sample after the CD4+ cells had been captured in a chamber using antibodies immobilized in a microfluidic channel. These are demonstrations where the sensing system was capable of using complex solutions such as human blood and providing useful information about the solution’s contents through the use of a sample preparation step.

*Nanopore Sensors for Single Molecule DNA Detection*

Nanopores, nanoscale analogues of Coulter counters, are devices that measure the ionic current between two sides of a nanoscale opening (1-50 nm). As DNA (~2 nm in diameter) translocates through the pore, the measured resistance between the two electrodes is heavily affected, inducing fast current pulses. For this to happen, the nanopore must be sufficiently small so that current blockage is observed as the DNA passes through the pore. Many nanopore reviews have been published (61-64). There are many attractive reasons to use nanopore sensors, including potential for increasing DNA sequencing read lengths, removal of DNA amplification steps, reducing reagent usage down to single molecule probing, and epigenetic studies of the effect of methylation on DNA transcription.

Various technological barriers must be overcome for nanopores to reach their full potential (65). For example, the DNA translocation times need to be increased from the 1-3 μs/nucleotide to values in the ms/nt range where sensitive electrical measurements can be performed to identify individual bases with high signal to noise ratio. In addition, the spatial resolution needs to be increased so that single nucleotides can be probed electrically. Also, the DNA extraction and
concentration steps need to be integrated with the nanopores so that small volumes or number of molecules can be used without the need for amplification.

Several schemes have been proposed to increase translocation times. Iqbal et al. demonstrated a solid state nanopore functionalized with hairpin loop DNA to increase the interaction of the translocated DNA with the pore(66). The electronics of the detection system have also been optimized to filter out high frequency noise and reduce the detectable translocation time limit(67). Venkatesan et al. demonstrated the use of aluminum oxide for nanopore membranes for improved stability with slower transport due to the positive surface charge and with higher SNR due to reduced noise properties(68; 69). Recently, companies including Oxford Nanopore Technologies, NABsys, and Genia, are working to commercialize nanopore technologies for sequencing applications. While many challenges lie ahead, electrically-based nanopores remain very attractive candidates as next generation sequencing technologies. Forming nanopores in electrically conducting graphene could allow for electrical or electrochemical measurements with very high spatial resolution as the thickness of single layer of graphene is about the same as the thickness of a single nucleotide(70).

**Affinity Based Sensors**

Affinity based biosensors rely on a functionalized surface with an immobilized probe molecule that can specifically capture the target analyte from solution. The kinetics and theory of receptor-ligand binding have been well studied with the Langmuir adsorption isotherm(71).

Impedance affinity based biosensors can be divided into Faradaic and non-Faradaic sensors. Faradaic impedance sensors monitor a change in the charge transfer resistance at the electrodes upon the binding of the target molecules(72). Faradaic DNA sensors have shown detection of 15mer DNA targets down to 1
nM(73) and detection of various proteins with aptamer probes(74; 75). A more recent report demonstrated the detection of microRNA (miRNA) down to 2 pM from various sources, including cancer cell lines and serum(76). Faradaic sensors run the risk of damage or change in properties of the surface probe molecules(72). Non-faradaic impedance sensors measure the capacitance component of impedance, since there is no charge transfer(77). As target molecules bind to the surface, the measured capacitance is modulated. Such sensors with electrodes on-chip usually use interdigitated electrodes (IDEs), where fingers of metal belonging to two distinct electrical nodes are patterned on-chip with periodic spacing. As the feature size of the electrodes is reduced to the comparable sizes of the relevant analytes, the sensor response is increased. IDEs have been used for detection of DNA in the nM range(78), DNA byproducts of PCR effectively down to the pM range(79), and antibodies in the pg/mL range(80). A recent work demonstrated a novel method for concentrating DNA by monitoring the impedance of a droplet containing DNA using a non-Faradaic technique as the droplet evaporated, which enabled detection of 60 attomolar initial DNA concentration(81). An on-chip working electrode can be combined with a solution reference electrode for capacitive measurements; however, incorporation of an on-chip reference electrode presents difficulties for mass manufacturing and packaging (77; 82).

Graphene has been utilized as a material for Faradaic impedimetric biosensing. Graphene has several attractive properties, including high metallic conductivity, high electron transfer rates, and large surface area(83). The inherent large surface roughness implies the potential to increase the density of immobilized probe molecules and the amount of captured target molecules. Li et al. demonstrated detection of SNPs down to the pM range using hairpin probes that were physically adsorbed to their graphene surface(84). Various works have also shown the use of graphene for electrical sandwich immunoassays for the detection of proteins including immunoglobulins and cancer biomarkers in the
nanomolar range (85; 86). However, the field of using graphene materials for sensing is still new. It is not proven that the properties of graphene translate to increased efficacy of sensing and a streamlined mass manufacturing technique does not exist.

**Perspectives**

Much has been reported in the last decade utilizing impedance-based measurements for biosensors. The core strength of this technology is the simplicity of the device fabrication. This has translated in some examples to more focus on integration of sample preparation modules with the sensors so that the overall system can input more complicated matrix solutions. We will evaluate impedance biosensors based on the following metrics:

- **Ease of device fabrication:** **high.** Very simple fabrication techniques can be used to construct the devices. In addition, no extra buffers or solutions (such as enzymes or labels) are typically needed for this technology.

- **Amenability for multiplexed detection:** **medium.** Several examples of the technology such as Coulter counters or nanopores exhibit limited capacity for multiplexing; however, affinity-based impedance sensors should allow for multiplexing by using specific capture probes.

- **Complexity of input solution:** **low-medium.** Most impedance sensors need a lower conductivity solution to operate so that the target analytes significantly influence the measured solution properties. There are a few works that have demonstrated cell counting from human blood, but these have incorporated significant sample processing steps (60).

- **Sensitivity:** **medium.** Most nucleic acid and protein detection is typically in the high pM to nM range, which is not significantly better than ELISAs or DNA microarrays (77).
- Commercialization status: **low-medium**. There are a few examples of commercialized impedance biosensors, mostly Coulter counters such as Daktari’s CD-4 counting platform. Several startups are trying to commercialize the nanopore technology, but no robust product has yet appeared on the market.

For impedance biosensors, the target entity must significantly affect the overall impedance of the solution, which requires large target entities, higher concentrations, or smaller interrogation volumes. In addition, more robust and repeatable surface functionalization procedures will be a key requirement for affinity-based impedance biosensors. In the future, impedance based techniques may be employed in niche POC applications such as global health, where portability and cost restrictions prohibit the use of standard optical techniques but better sensitivity or multiplexing capabilities are not critical requirements.

### 3.2 Potentiometric Biosensors

When any electrode surface is placed in contact with an ionic solution, a potential drop forms at the surface ($\psi_0$), which is a strong function of surface charge. When an affinity based biosensor captures charged target analyte molecules on a surface, $\psi_0$ is affected. A potentiometric biosensor uses the change in surface potential at an electrode surface to detect the presence of biomolecules. Potentiometric biosensors offer the possibility of increased specificity and multiplexed detection due to affinity based capture schemes when compared to impedance or amperometric sensors. In addition, flexibility with sample volume is afforded by these techniques since the induced potential changes occur right at the surface of the biological interface. The requirement for impedance biosensors of small sample volume or large target entities can therefore be avoided with this technology. However, potentiometric sensors are typically more complicated to fabricate, functionalize, and measure. In particular, the issue of achieving stable and repeatable surface chemistries for affinity based biosensors remains a field
with significant opportunity for standardization and optimization. In addition, target entities must be present within a Debye length of the surface to significantly induce sensor response.

*Electrolyte Insulator Semiconductor Systems*

Electrolyte Insulator Semiconductor (EIS) systems, when combined with a reference electrode immersed in solution, form a structure similar to the fundamental two terminal Metal-Oxide-Semiconductor (MOS) capacitor, with the metal gate replaced by the reference electrode and the ionic fluid. As charged target molecules bind to the surface, the charge induces an opposite charge split into two components: the counter-ion cloud in the double layer, and carriers in the semiconductor. This can be modeled as two capacitances in parallel, the double layer capacitance $C_{DL}$, and the device capacitance $C_{device}$. The voltage across these capacitors is $\psi_0$, and the change in $\psi_0$ due to the binding of the target on the surface will shift the capacitance-voltage (CV). The charge at each capacitor interface is determined by the relative magnitudes of the two capacitors; the larger capacitor will mirror most of the charge of the target molecules. $C_{DL}$ increases as the ionic strength of the solution increases, robbing the device of transducer charge and decreasing overall sensor response. Works have shown detection of various molecules with EIS capacitor structures down to the nM range (87-89), but most of the work in the field has focused on field effect transistors for biological detection.

*Nanoscale Field Effect Sensors*

Ion Sensitive Field Effect Transistor (ISFET) sensors are three or four terminal devices that add source and drain terminals to the EIS capacitance structure. Here, the output characteristic is the current measured between source and drain ($I_{DS}$). Commercial pH sensors have been produced using the ISFET technology. The main advantages of a FET structure include the intrinsic device gain of a
transistor and the ease of current measurements in large arrays. Many reviews have been published covering such sensors (90-92).

NanoFET sensors are ISFETs that have been scaled down to the nanoscale in thickness and width. By reducing the size of the devices close to the order of targeted molecules, the LOD can be reduced. In a landmark publication, researchers used silicon nanowires 20 nm in diameter formed using bottoms-up vapor-liquid-solid (VLS) growth, a method not suitable for scale up (93). The devices were used for applications detecting DNA and proteins down to the fM range (50; 54). Stern et al. used a top down fabrication process with electron beam lithography to create silicon nanowire FETs (25 nm thick, 50 nm wide) from silicon-on-insulator (SOI) wafers (94). Detection down to 10 fM of streptavidin and 100 fM of mouse immunoglobulin A was demonstrated. Another group has published the specific detection of microRNA with top down fabricated nanowires using PNA probes down to 1 fM (95), multiplexed detection of the cardiac biomarkers from human serum down to 100 fg/mL (96), and detection of carbohydrate-protein interactions down to 1 fg/mL (97). Dorvel et al. demonstrated specific miRNA detection from buffer down to 100 fM using hafnium oxide as a high-k dielectric for silicon nanowire sensors 50 nm in width and 30 nm thick. The use of a high-k dielectric allows for a thicker gate oxide without sacrificing the gate oxide capacitance. A method has also been illustrated for transferring nanowire FETs to plastic flexible substrates (98). Carbon nanotubes have also been used as FET biosensors, due to their biocompatibility and high theoretical sensitivity since all of the atoms of carbon nanotubes are present at the surface (99). Graphene has been used as a sensing membrane for FET sensors due to its high conductivity and atomic layer thickness (100), but there are still several issues with this young field, including standardization of preparation and surface conjugation difficulties.
There are few reports of FET sensors demonstrating sensing from complex matrix solutions, due to three main obstacles. First, the large amount of charged entities in more complicated samples can lead to an abundance of false positives without high specificity of the capture probe. Increasing salt concentration and optimizing the pH of the solution can improve specificity. Second, surface receptors can degrade rapidly with exposure complex matrices such as blood or serum(92). Third, physiological solutions have ionic strength of around 100 mM, which corresponds to a Debye length of 0.7 nm. As charged entities are further away from the surface, this charge will be screened by ions, which will minimize induced electric field in the sensor. For reference, a monolayer of typical silanes used as the first anchor layer in all commonly used oxide surface functionalization schemes is around 0.8 nm thick(101). Most schemes will therefore anchor charged targets far beyond the Debye length in a physiological solution(102). To alleviate this issue, most researchers use diluted blood or sensing buffers with low ionic strength, since the Debye length is inversely proportional to the square root of ionic strength. However, molecular affinity and specificity is reduced without stabilizing salts. Either device sensitivity (high salt, low Debye length) or device specificity (low salt, low probe affinity) must be sacrificed. Significant sample preparation steps will be needed to transform complex sample inputs such as blood into low ionic strength buffers that allow the devices to sense target entities.

To address this issue, the use of a microfluidic purification chip (MPC) for detection from whole blood was proposed (53). Human blood is flown into the MPC, which contains immobilized antibodies specific to capture the target biomarkers to be detected. After washes to reduce the ionic strength of the solution, the target biomarkers are released and detected by the nanowire chips in a low salt solution to maximize signal strength. Specific detection was demonstrated from human blood spiked with as low as 2.5 ng/mL of prostate specific antigen (PSA) and 30 units/mL of carbohydrate antigen (CA) 15.3. Makowski et al. offer a summary of other works that claim detection from blood
and serum(92), but several of these schemes employ labels or fail to address the issue of ion screening at all.

FETs have also been used to monitor the byproducts of chemical reactions on-chip. Ion Torrent, a next generation sequencing (NGS) company, described an integrated semiconductor device with completely non-optical sequencing(103). Their chip (<$100) has over 1.2 million sensors integrated with on-chip multiplexing and signal processing. PCR beads containing the fragments of the template DNA to be sequenced are loaded into individual wells covering each sensor in a row-column array, and sequencing by synthesis is performed. As each of the four nucleotides is introduced sequentially to the wells, polymerase adds bases to the nascent strand, which hydrolyzes the incoming nucleotide’s triphosphate, producing hydrogen ions that can be detected by the underlying FET sensors. Much of the Ion Torrent intellectual property has been licensed from a POC care company called DNA Electronics. DNA Electronics combined on-chip signal processing, control circuitry, on-chip heaters and temperature sensors for the detection of pH changes induced by LAMP (104). This technology is intended as a non-optical replacement for quantitative PCR, the current standard for genotyping experiments.

In addition to the difficulties of detection from physiological solutions addressed previously, the FET technology faces standardization issues (92; 105) and mass fabrication issues. There are very few works to date utilizing foundries for the direct label-free sensing of target analytes. At the heart of this problem is the issue of device geometry - most specifically the question of the necessity of nanostructured devices. A survey of published works according to their stated device width and thickness is shown in Figure 2.5, with relevant sensing parameters in Table 2. Sections of width and thickness have been delineated according to different difficulties of fabrication. For example, any device with width below 70 nm requires specialized techniques such as electron beam
lithography or bottoms up growth. Few works have focused on the Standard SOI Foundry Processing region of this graph. It will be important to determine the resolution and dynamic range that are needed for the target applications to see if foundry fabricated sensors can meet these specifications.

Theoretical frameworks have attempted to address this issue (106-109). They argue that device geometry and detection time are inextricably linked. For example, diffusion-limited transport of a target analyte to the sensor is a strong

![Figure 2.5 Dimensions of FET Sensors](image)

*Figure 2.5 Dimensions of FET Sensors:* A survey of FET sensor works is shown, organized according to the reported device thickness and width. Widths below 70 nm require specialized fabrication techniques. Thicknesses below 100 nm are difficult to produce with foundry processing. Most reported works use nanowire devices with <70 nm in width and <50 nm in thickness. Volume inversion, where the entire physical device is enriched with carriers, should yield optimal sensitivity, though no reported works lie in this regime.
function of device width; the density of analytes on a planar structure is proportional to the square root of time, compared to a linear relationship for nanowire structures\(^{(106)}\). However, nanostructured sensors have lower LODs due to less charge contained in the active layer. This model predicts that given a 100 second detection time, the LOD should be around 1 pM for nanowires and 100 pM for planar ISFETs. They argue that the fM detection limits shown by various works are actually statistical anomalies from the tail ends of distributions of device measurements. It will be important for groups to provide statistical distributions of detection events instead of illustrating the results from single devices.

<table>
<thead>
<tr>
<th>Nu.</th>
<th>Molecule(s)</th>
<th>LOD</th>
<th>Solution</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA</td>
<td>fM</td>
<td>Low ionic stren.</td>
<td>(50)</td>
</tr>
<tr>
<td>2</td>
<td>PSA, CEA, mucin-1</td>
<td>fM</td>
<td>1 μM phosph buff</td>
<td>(54)</td>
</tr>
<tr>
<td>3</td>
<td>streptavidin</td>
<td>fM</td>
<td>1 mM sodium bicarbonate buffer</td>
<td>(94)</td>
</tr>
<tr>
<td>4</td>
<td>PSA, CA 15.3</td>
<td>pM</td>
<td>Human blood</td>
<td>(53)</td>
</tr>
<tr>
<td>5</td>
<td>miRNA</td>
<td>fM</td>
<td>0.02X SSC</td>
<td>(110)</td>
</tr>
<tr>
<td>6</td>
<td>miRNA</td>
<td>fM</td>
<td>0.01X SSC</td>
<td>(95)</td>
</tr>
<tr>
<td>7</td>
<td>cTnT, CK-MM, CK-MB</td>
<td>fM</td>
<td>Human serum</td>
<td>(96)</td>
</tr>
<tr>
<td>8</td>
<td>cTnI</td>
<td>fM</td>
<td>0.01X PBS</td>
<td>(111)</td>
</tr>
<tr>
<td>9</td>
<td>DNA</td>
<td>fM</td>
<td>0.1X PBS</td>
<td>(112)</td>
</tr>
<tr>
<td>10</td>
<td>DNA</td>
<td>μM</td>
<td>1 mM phosph buff</td>
<td>(113)</td>
</tr>
<tr>
<td>11</td>
<td>BSA</td>
<td>nM</td>
<td>1.5 mM phosph buff</td>
<td>(114)</td>
</tr>
</tbody>
</table>

**Table 2.2 FET Sensing Overview**

**Perspectives**

The works by Ion Torrent and DNA Electronics exemplify the power of potentiometric sensors. The scalability and versatility of the semiconductor manufacturing process available in foundries such as Taiwan Semiconductor
Manufacturing Corporation, Samsung foundries, and Global Foundries can translate into massively multiplexed biochips. We will evaluate potentiometric biosensors based on the following metrics:

- Ease of device fabrication: low. Particularly with the FET sensors, fabrication is very complicated and is still quite expensive. However, after initial barriers have been surmounted, this technology has capacity for mass fabrication to reduce unit costs. The lack of a label is an attractive feature of FET sensors, reducing overall cost and complications associated with modifying target analytes.

- Amenability for multiplexed detection: high. The possibility for large arrays of FET devices with many different target analytes on the same chip offers the most attractive potential for multiplexing out of all the technologies covered in this review. Ion Torrent has shown that thousands of different reactions can take place on a single chip. However, multiplexed intrinsic molecular sensing has yet to be demonstrated with a commercial device.

- Complexity of input solution: low. The most problematic dilemma of the FET technology is the tradeoff between sensitivity and selectivity mentioned previously. Most sensors require highly purified buffer solutions with low ionic strength. Until this problem is adequately resolved, significant sample preparation will be needed.

- Sensitivity: high. Demonstrated LODs have been in the fM or even aM range, far below standard techniques. However, some issues still exist in interpretation and standardization of this data.

- Commercialization status: low-medium. Ion Torrent and DNA Electronics have used FETs for commercial products. However, this has used the pH changes of polymerase reactions for detection. Thus far, no companies
have a commercial product for intrinsic molecular detection, though many startups are attempting to do so.

In the future, potentiometric sensors offer the potential for low per unit sensor costs, massive multiplexing, and very low limits of detection. However, a significant lack of standardization of fabrication techniques, surface functionalization, and measurement schemes has prevented the field from full maturation. One of the most significant barriers that must be surmounted is the requirement for low ionic strength solutions to increase the Debye length and reduce charge screening. FET sensors may replace DNA microarrays and ELISAs with devices that are cheaper, more portable, have higher sensitivity, and require smaller sample volumes for many applications including drug discovery, early cancer detection, food safety, and global health.

3.3 Amperometric Biosensors

The research in amperometric biosensors dates back to the 1960s (115), making it a very mature field in the world of biosensors with thousands of published papers. Amperometric biosensors take advantage of enzyme-driven redox reactions to specifically detect targeted biomolecules in solution. In this method, an enzyme that catalyzes the generation of redox reaction components can be free in solution, immobilized at an electrode surface, or immobilized in a matrix that also contains the necessary mediators. The introduction of a medium containing substrates subject to the enzyme’s catalytic behavior causes a reaction and generates a product, which is then oxidized at the surface of an integrated electrode. This results in electron generation and a faradaic current in the underlying electrodes—the latter of which is measured and then correlated to the concentration of the targeted biomolecule. Of all the technologies covered in this review, amperometric sensors are the most widespread, most likely due to the simplicity and repeatability of the technique. The measurement is executed using two electrodes that simply measure a Faradaic current generated in the presence of the
enzyme, substrate, and mediators. However, the technique can only be used with very specialized targets where there is a known enzyme that can catalyze a reaction to produce changes in the detected current. As of today, this limits the applications amperometric sensors can be used for to a minute subset of biological targets.

Background

Blood glucose sensors represent the gold standard for amperometric biosensors and potentially all biosensors. This technology has directly impacted diabetics on an everyday basis and served to introduce the potential of biosensors to the world. Given its ubiquity, the glucose meter serves as a sound starting point for introducing the fundamentals behind amperometric biosensors.

Amperometric glucose sensors monitor the current glucose concentration in a droplet of patient’s blood. Traditionally, this method utilizes glucose oxidate-catalyzed reaction, which generates hydrogen peroxide. A subsequent electrical signal is measured from the oxidation of hydrogen peroxide or reduction of oxygen. To accurately measure the current generated, a three-electrode system is utilized consisting of a reference, working, and counter electrode. A stable potential is maintained with the reference electrode vs. working electrode, while current is measured between the working electrode and the counter electrode. To maintain the stability of the reference electrode, no current should be allowed to pass through this electrode.

\[
\text{Glucose} + \text{GOx (FAD)} \rightarrow \text{Gluconic acid} + \text{GOx(FADH}_2\text{)}
\]

\[
\text{GOx(FADH}_2\text{)} + \text{O}_2 \rightarrow \text{Ox(FAD)} + \text{H}_2\text{O}_2
\]

\[
\text{H}_2\text{O}_2 \rightarrow 2\text{H}^+ + \text{O}_2 + 2\text{e}^-
\]
This method can be extended to a wide variety of other analytes, as long as an enzyme can be found that will result in an oxidation-reduction reaction and the subsequently generated electrons sensed. In order to sensitively detect the generated electrons, it is important that the enzyme be localized at the electrode surface, which is often accomplished through immobilization techniques.

Figure 2.6 Three Generations of Amperometric Biosensors: (A) The *first generation* of amperometric biosensors relied on generation of an electroactive by-product such as hydrogen peroxide to generate a measureable current through oxidation/reduction at the electrode. (B) The *second generation* introduced mediators to move electrons from the enzyme to the electrode. (C) The *third generation* removed the need for by-products and mediators entirely by utilizing direct electron transfer from the active site of the enzyme to the electrodes. (D) A *variation of the third generation* utilizes nanostructures to directly transfer electrons to the electrodes by bringing conductive elements closer to the active site of the enzyme.
To date, there have been three generations of amperometric biosensing modalities that each use the redox reaction in their own unique way (116). As depicted in Figure 2.6A, the first generation resembles the reaction shown above, where electrons are sensed from an enzyme’s byproduct oxidation/reduction. This method offers simplicity, but if the byproduct is naturally occurring in the sample (like oxygen is for glucose sensors), variations in oxygenation of blood can cause large background noise and reduced resolution. The second generation, Figure 2.6B, bypasses this problem by using a molecular mediator, such as ferrocene for glucose sensors, to mediate electron transfer between the enzyme cofactor and the electrode. Figure 2.6C depicts the most recent generation. This new sensing modality relies on strictly direct electron transfer from the active site of the enzyme to the electrode surface (116). This can greatly reduce complexity of the required reagents and increase specificity, but larger distances (>8 angstroms) of the active site to the electrode can significantly reduce the rate of electron tunneling and therefore sensitivity. This method has seen limited use in glucose sensors, as glucose oxidase’s active site is centrally located and is at least 13 angstroms from the enzyme’s edge (117). To overcome this issue, researchers have turned to nanostructures to increase the conduction of electrons from the active site to the electrode. As shown in Figure 2.6D, carbon nanowires bring the electrode closer to the active site of the enzyme. Alternatively, conductive nanoparticles can be bound to the enzyme, which can increase the electron transfer rate to nearby electrodes. These systems have not been implemented in commercial devices yet, but initial results are promising.

Research Examples

Even though amperometric biosensors were invented more than 50 years ago, new innovations continue to improve the sensor performance. Today, and as depicted in Figure 2.6D, the innovations primarily revolves around 2 areas: the incorporation of nanotechnology and development of the third generation of
amperometric biosensors that utilize direct electron transfer modalities. Nanotechnology can be incorporated in three major ways: (i) modification to macroelectrodes with nanostructures such as nanoparticles, nanopillars or carbon nanotubes, (ii) incorporation of nanoelectrodes (thickness or width), and (iii) modification of biomolecules with nanomaterials such as nanoparticles or nanotubes.

Modification of macroelectrodes with nanostructures has become increasingly popular in recent years. Specifically, carbon nanotubes (CNTs) have seen a variety of uses including, but not limited to, detection of cancer biomarkers(118), glucose(55; 119), lactate(57), glutamate(120), horseradish peroxidase(121), and bisphenol-A(122). Carbon nanotube sensors improve sensitivity of detection by increasing the surface area available as well as potentially altering the activity and electron transport of the targeted biomolecule by bringing the electrode surface closer to the enzyme’s active site.(123; 124) Utilizing nanoelectrodes also offers unique advantages to amperometric biosensors as shown recently with the detection of glucose(56; 125) and L-dopa(126). The increased surface area and small dimensions of nanoelectrodes have been shown to increase the sensitivity and enhance mass transport by utilizing hemispherical diffusion fields in amperometric biosensors.(127) Modification of biomolecules with nanoparticles is an emerging field. As the understanding of enzyme function continues to grow through improvements in folding simulations and sequencing, nanotechnologists will be able to modify proteins to increase activity and the rate of electron transfer from the active site. (128-130) Meredith et al. recently demonstrated the modification of multiwalled carbon nanotubes with anthracene to aid orientation of laccase.(128) In doing so, they brought active site closer to the electrode, which allowed for greater direct electron transfer.

Towards in vivo Electrochemical Sensors
Amperometric biosensors that are integrated with the human body enable continuous and less invasive monitoring of biologically relevant components, such as glucose and lactate. Commercially, a new class of glucose sensors utilize device placement just under the skin. These systems work by monitoring glucose levels in interstitial fluid and can simplify the management of diabetes through implementation of a minimally invasive, continuous monitoring system. Additionally, sensors can be placed on the skin or on a contact lens for continuous monitoring of biomolecules through sweat or tears.(131) Even though the majority of research has focused on glucose monitoring for diabetics, progress has also been made in monitoring lactate. Jia et al. designed an electrochemical sensor system to be worn as a temporary tattoo—complete with working, counter, and reference electrodes and an included enzymatic layer (58). The enzymatic layer included lactate oxidase, which converted lactate from sweat into pyruvate and, in the process, generated electrons for an amperometric measurement. Systems such as these can be adopted to monitor a wide variety of biomolecules in a minimally invasive manner, and when placed on the skin can bypass many biocompatibility issues associated with implantation.

Perspectives

Amperometric biosensors present a unique opportunity in the world of biosensors. Their simple design and large background of research provides an intriguing arena for biosensor development. We will evaluate amperometric biosensors based on the following metrics:

- Ease of device fabrication: **high**. First and second generation sensors utilize a simple design of interdigitated electrodes. Their cost is only slightly increased due to the need for incorporated enzymes and mediators.
- Amenability for multiplexed detection: **low**. The specific nature of the enzyme catalyzed reaction limits the potential for detection of multiple analytes.
• Complexity of input solution: **high**. The specific nature of the reaction as well as the inclusion of reaction mediators allows commercial amperometric biosensors to utilize complex matrices such as blood, tears, and saliva.

• Sensitivity: **low**. Typical detection limits are in the µM range. This provides relevant data for some analytes, but it is not sensitive enough for many applications such as typical DNA and cancer biomarker detection.

• Commercialization status: **high**. The ubiquity of blood glucose sensors is a prime example of the commercialization of amperometric biosensors.

The amperometric biosensor is limited by its multiplexing capability and sensitivity. Third generation sensors that utilize (i) direct electron transfer to eliminate mediators and increase multiplexing capability and (ii) nanotechnology to increase sensitivity will address these issues, but their commercialization prospects are still limited. Further standardization of direct electron transfer measurement techniques as well as a greater understanding of nanomaterial fabrication and enzyme structure/kinetics is necessary for this field to mature. Until third generation sensors develop further, applications will continue to focus on scenarios where measurements must be taken on a regular basis, but the sensitivity can be limited to the µM range, such as measurement of contamination of groundwater from arsenic or nitrates.

4 **Future Perspectives**

Electrical biochips have shown a great deal of promise in the last decade, and will continue to grow in importance in coming years. Electrical devices are highly attractive for POC diagnostics due to lower cost, ease of integration, and potential for portability. The POC diagnostics market reached $13.4 billion in 2010, and is expected to grow to $16.5 billion in 2016(8).

4.1. **Current Trends and Unmet Needs**
Commercial Products

By far the most successful commercial biosensor has been the amperometric glucose sensor. Glucose test strips for home use are produced on a massive scale, approaching $10^{10}$ strips produced per year (7). Various behemoths in the diagnostics industry have commercial products, including Roche Diagnostics (Accu-Chek Systems), Johnson and Johnson (LifeScan Meters), Abbott Diabetes Care (FreeStyle System, Precision System, i-STAT), and Nipro Diagnostics (Sidekick and TrueTrack Systems). These portable systems have enabled an entirely new level of at-home personal care, increasing the freedom of millions of diabetes patients across the world. These sensors satisfy virtually all the ideal checklists for POC sensors, including fast time to results (~5s), small sample volumes (down to 300nL), cheap per-test costs, and a lack of the need for technical expertise for operation (7). The latest systems, such as Abbott’s Freestyle Navigator, MedTronic’s MiniMed Real-Time Revel Paradigm, or Dexcom’s Q4 Platinum, enable continuous glucose monitoring with insertion of an electrode just underneath the skin. Similar technologies are used for analyzing blood chemistry, cardiac markers, cholesterol levels, and urine chemistry. These technologies are examples of the potential of POC devices in the health care industry; however, use of this specific technology to a variety of different applications is quite limited due to the specific nature of the enzymatic reaction. Some concerns exist about the accuracy of detection from POC sensors (132) and the efficacy of electrical POC devices to standard techniques for making treatment decisions, with a focus on Abbott’s versatile i-Stat system due to its ubiquity (133; 134). The overall conclusion is that there is a significant opportunity to improve the accuracy of POC devices to align with currently used techniques.

Academia to Industry
There is a large gap between the huge academic output of biosensor work and commercial successes (135; 136), due to many reasons. First, more focus needs to be placed on detection in complex matrix solutions. Specifically, better standardization of metrics such as LOD, resolution, and dynamic range need to be quantified in solutions such as human blood, serum, urine, or saliva. Such frameworks are already well established by institutes such as the Clinical and Laboratory Standards Institute (CLSI), a non-profit organization that is one of the primary authorities on unified standards for various lab practices in health care. For example, LOD is properly determined by using statistical analysis of the response of many samples, including proper determination of the limit of blank (LOB) in the application’s complex matrix solution. Most published biosensor works opt instead to demonstrate traces from only a few sensors in pure buffer. Second, more focus on sample preparation and the elements surrounding the sensor component is needed. POC sensors are not functionally useful if they require considerable off-device preparation with expensive laboratory equipment or a skilled technical staff. Third, surface conjugation techniques for affinity-based biosensors need to be optimized and standardized. A systematic comparison between various approaches is needed, with clear specification goals including achieving the highest density of molecules, high specificity to the target analyte, and other technology specific requirements such as the closest attachment to the surface. Finally, better analytical and simulation studies will be needed to more fully understand the complexity of surface conjugation to affinity-based biosensors. All of these issues will need to be addressed by universities, start-up companies, and industry leaders to attempt to close the gap between academia and industry in the coming years.

Global Health and Resource Limited Settings

The development of POC devices for resource-limited settings has become a very important goal for the world in the 21st century(7; 137). Substantial funding
has been poured into this area, from various organizations, including the Bill & Melinda Gates Foundation and the U.S. Global Health Initiative. Most of the attention has focused on infectious diseases, including HIV, tuberculosis (TB), and malaria. In contrast to resource-rich settings, where portable, cheap devices requiring minimal technical expertise and laboratory equipment are more of a desirable luxury than an absolute necessity, in resource-limited settings such devices are often the only option. For these settings, simplicity and cost are the two most urgent considerations. Electrical POC biochips have been used for many of these devices to satisfy these requirements, most notably with technology similar to the glucose sensor and with impedance measurements in fluidic devices. Global health will continue to be a key niche for electrical biochips in the coming years.

**Personalized Medicine and Biomarkers**

The rise of cheaper and faster sequencing techniques in the last decade has given rise to a new paradigm for health care called personalized medicine, where treatment strategies are tailored to a patient based on their individual available biomolecular information – in particular, the concentration of relevant biomarkers. The discovery of new biomarkers can help both in the never ending quest for gaining a more complete understanding of disease pathways and in drastically improving patient survival rates by enabling early detection of disease. Biomarkers are becoming increasingly important for oncology, neurogenerative diseases, cardiovascular diseases, autoimmune and inflammatory disorders, traumatic brain injury, and prenatal screening/inherited disorders.

Semiconductor fabrication allows for massively scaled multiplexing so that a huge amount of different biomarkers can be tested for with a large amount of redundancy. Redundancy is essential since the number of measurements is directly correlated to resolution and limits of detection. Affinity-based electrical biochips may evolve into versatile plug and play platforms, where the same
technology can apply to a large variety of analytes, all based on the probe-target capture model. In addition to these factors, the other advantages of low cost, portability, lower sample volume, faster response times, and elimination of labels will ensure that electrical biochips occupy a significant portion of the biomarker detection market.

4.2. Future Outlook

As we move into the future, the trend will be for biosensors to move away from heavily equipped research laboratories and towards more distributed use in hospitals, doctor’s office, or at homes. Both in vivo and in vitro biosensors will exist for a range of applications. Sampling from breath, skin or body fluids could be used to categorize the in vitro sensors as the source of the sample can drive the technologies to be used. Long-term in vivo sensors still remain a grand challenge with new flexible sensors that are mechanical compliant with specific organs as an important new advancement for in vivo applications, especially for short term use during surgical procedures, for example. In addition, therapeutics may be integrated with the diagnostics components as a real-time feedback-sensitive treatment option, an emerging field known as theranostics. The seamless integration of electrical techniques with biological processes seems inevitable, and many important steps have been taken towards this ambitious and fruitful goal.
CHAPTER 3

ULTRA-LOCALIZED THERMAL REACTIONS OVER SILICON FIELD EFFECT TRANSISTORS

Abstract

Miniaturized lab-on-chip systems promise rapid, sensitive, and multiplexed detection of biological samples for medical diagnostics, drug discovery, and high throughput screening. Within miniaturized ‘lab-on-chips’, static and dynamic droplets of fluids in different immiscible media have been used as individual vessels to perform bio-chemical reactions and confine the products. However, not reported before are approaches to perform localized heating of these individual sub-nanoliter volume droplets, a capability that can allow for new applications that require parallel, time- and space-multiplex reactions on a single integrated circuit. Our method positions droplets on an array of individual silicon microwave heaters on chip to precisely control the temperature of droplets-in-air, allowing us to perform biochemical reactions, including DNA melting and detection of single base mismatches. We also demonstrate that ssDNA probe molecules can be placed on heaters in solution, dried, and then rehydrated by ssDNA target molecules in droplets for hybridization and detection. This platform enables many applications in droplets including hybridization of low copy number DNA molecules, lysing of single cells, interrogation of ligand-receptor interactions, and rapid temperature cycling for amplification of DNA molecules.

This work has been published as Reference [(138)].
Introduction

Recent developments in high-throughput screening technologies have made it possible to process thousands of individual reaction volumes at a time (139). Previous sub-nanoliter screening techniques utilized droplets-in-oil, micromachined chambers, and other strategies (140-142). Encapsulating droplets with mineral oil, capping them with PDMS or covering and sealing microchambers with glass and a nail polish solution have all been used to minimize evaporation (141; 143-145). Similarly, biologically compatible solvents with low volatility have been used for limiting evaporation in parallel reactions for screening applications (146). Further integrating a miniaturized heating element with droplet screening technologies can enable many temperature mediated biochemical reactions such as high-throughput melting curve analyses of individually generated sub-nanoliter droplets. Such individually addressable heating elements at the microscale can allow for greater spatial and temporal control of temperature profiles.

Previous on-chip, localized heating designs focused on peltier heaters, resistive heaters, or other methods (147-151). A variation on the resistive heater uses a transistor as a heater whereby adjusting the source-drain current via modulation of the gate voltage can result in heating of the fluid above the device (152). This approach, however, required a very wide gate region (~700 μm) and is incompatible with the use of picoliter scale droplets. A second approach involving microwave heating of picoliter droplets in a microfluidic device has been studied (153) but this method does not allow for individualized heating of droplets and also requires mineral oil as an encapsulation layer to minimize evaporation. Finally, optical heating methods have also been employed (154; 155), but suffer from setup complexity for individually heating multiple droplets, and similarly require an encapsulation layer to minimize evaporation.

Earlier, we demonstrated heating via use of individual transistors by
applying an AC voltage at 10MHz and 10-25 V\textsubscript{rms} between the transistor’s leads and the bulk substrate of a silicon-on-insulator micro-ribbon transistor structure\textsuperscript{(3; 4)}. This technique focused on characterization of temperature profile at the surface of a device in a bulk fluid and did not offer control of thermal cross-talk and by-product diffusion between heating elements. To allow for individual reaction volumes, simple placement of droplets on heating units, minimization of evaporation without an encapsulation layer, reduction of thermal cross-talk, and elimination of by-product diffusion between heating elements, we have developed a droplet-in-air method using a low evaporation, biocompatible solvent (NanoInk, Skokie, IL) (see Figure 3.1). In the past, techniques using silicon wells with 0.4nL volumes with ethylene glycol to limit evaporation when exposed to air have been reported\textsuperscript{(156)}. Similar techniques using glycerol as the low volatility solvent showed longer droplet stability, but with temperature was limited to 37°C\textsuperscript{(146)}. In our system, individually addressable, spatially multiplexed, droplets were heated to above 80°C over the course of a few minutes without any noticeable evaporation.

**Methods**

*Chip Fabrication*

The fabrication flow and preparation of devices as well as techniques for heating and imaging were presented previously\textsuperscript{(4)}. A CMOS compatible top-down fabrication procedure was followed to create devices in silicon-on-insulator (SOIs) wafers. The wafer’s device layer was thinned down to ~300 Å by timed dry oxidation followed by buffered oxide etch. Active areas were lithographically defined and the rest of top silicon was etched using deep reactive ion etch (DRIE). Afterwards implant areas were defined with photoresist mask for Boron ion implantation. After doping, around 300 Å of silicon oxide was grown to form the gate oxide. Metal contacts (200 Å titanium/800 Å platinum) were patterned via lift off after wet etch removing of silicon oxide on top of contact regions. Finally,
a 5000 Å nitride rich PECVD passivation layer was deposited and patterned to expose device channel and probing pads. Resulting devices were 300 Å thick with a channel that was 10 μm long and 2 μm wide.

**Figure 3.1 Device and Methodology Schematic:** (A) cross-section of device with a droplet is shown. The left side shows an unheated droplet with the DNA FRET construct in the double-stranded form. The right side shows a heated droplet where the FRET construct has denatured resulting in an increase in fluorescence. (B) A microcapillary pressure injection system is used to spot droplets on individual devices. An AC signal between the shorted source-drain and the backgate of a device is used to heat the droplet. (C) A top-view of ~225 pL droplet placed on a heating element. The heating element is 2 μm wide in a 20 μm x 20 μm release window. Scale bar, 100 μm. (D) An array of droplets is spotted on linked devices. 11 linked on left module and 11 linked on right module. Scale bar, 100 μm
**Chip Preparation**

The chip surface was coated with a hydrophobic silane monolayer. Trichloroperfluoro-octyl silane (PFOS) was vapor deposited on the chip surface. The chips were first cleaned using an acetone, methanol, DI rinse and then oxygen plasma clean for 5 minutes at 300W. The chips were then placed in a desiccator inside a pyrex petri dish with 20 μL of the PFOS. A vacuum was pulled on the desiccator for 20 minutes to allow for vapor deposition of the silane on the device surface. After deposition, the devices were cleaned of excess using an acetone, methanol, DI rinse. Any remaining silane excess was removed using a microfiber swab.

**FRET Construct Solution Preparation**

Single stranded FRET constructs were ordered from IDT already lyophilized (sequences in Table 3.1. The DNA was rehydrated to a concentration of 50 μM using nuclease-free DI water. The ssDNA FRET construct was then mixed in equal parts to its complementary ssDNA FRET construct. To ensure proper hybridization, the mixture was heated to 95°C in a thermocycler for 150 seconds and then allowed to slowly cool to room temperature over the course of 5-10 minutes. To make each of the FRET construct solutions for droplet generation, 1 μL of the 25 μM dsDNA FRET solution was added to 10 μL of Protein Carrier Solution from NanoInk and 10 μL of 3X SSC buffer. Macroscale melting temperature readings were taken using an Eppendorf Realplex Thermocycler. These melting temperatures were used for comparing the melting voltage vs. the melting temperature for the different FRET constructs.
Microinjection procedure

Injection of the sub-nanoliter droplets was accomplished using a microcapillary pressure injection system typically using for Intracytoplasmic sperm injection. A microcapillary from TransferTip® F (ICSI), from Eppendorf, with a 7μm inner diameter, 15μm outer diameter tip was used. The tip was inserted into a holder that connected the tip to a Narishige IM-300 pressure regulation system. The microcapillary tip holder was inserted into a 3D motorized micromanipulator system (Ultraprecise Motorized Micromanipulator from Warner instruments). This system has 10nm resolution with a range of 10 mm and allows accurate manipulation of the microcapillary tip for droplet placement. A Leica upright microscope was used for device visualization during droplet placement. The FRET construct, mixed with low evaporation solution, was loaded into the tip by suctioning for 2 seconds. The tip was positioned above the chosen device active

<table>
<thead>
<tr>
<th>Table 3.1 Sequence and melting temperature of the FRET constructs:</th>
<th>This provides the sequence information and dye modification for each of the FRET constructs used. The melting temperatures were determined using a commercial real-time PCR system’s melting curve protocol. The single base mismatch in the (72-70°C) Heteroduplex is highlighted in red.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sequence</strong></td>
<td><strong>Measured melting temperature (T_m)</strong></td>
</tr>
<tr>
<td>1</td>
<td>5’-6-FAM/TGGATCCATAGGTAG-3’ 3’-IBAKFO/TTTTTTTTTTTACCTAGGTACATC-5’</td>
</tr>
<tr>
<td>2</td>
<td>5’-6-FAM/TGGATCCATAGGTAGCT-3’ 3’-IBAKFO/TTTTTTTTTTACCTAGGTACATC-5’</td>
</tr>
<tr>
<td>3</td>
<td>3’-6-FAM/TTTTTTTTGAGACGGCAGCGGT-5’ 5’-IBAKFO/TCGCTGGCTTCGTGCAAGGCA-3’</td>
</tr>
<tr>
<td>4</td>
<td>5’-IBAKFO/GTTGATGTTAGCTGGTGTCATAG-3’ 3’-6-FAM/TTTTTTTTTTTCAACTACATCGCACAGTAAT-5’</td>
</tr>
<tr>
<td>5</td>
<td>5’-IBAKFO/GTTGATGTTAGCTGGTGTCATAG-3’ 3’-6-FAM/TTTTTTTTTTTCAACTACATCGCACAGTAAT-5’</td>
</tr>
<tr>
<td>(4-5) Heteroduplex</td>
<td>5’-IBAKFO/GTTGATGTTAGCTGGTGTCATAG-3’ 3’-6-FAM/TTTTTTTTTTTCAACTACATCGCACAGTAAT-5’</td>
</tr>
</tbody>
</table>
area and the solution injected for .01-.03 seconds, which resulted in a 50-100 μm diameter droplet with an estimated volume of hundreds of picoliters. The tip was then moved to the next device using the motorized micromanipulator where the injection procedure was repeated.

*Heating procedure*

RF dielectric heating, described previously(3), was induced in each device by applying an AC bias between shorted drain/source (or only source in ‘common-source’ experiments) and silicon substrate. Using double sided adhesive conductive carbon tape (SPI supplies), the chips were adhered to a brass plate that acted as chuck to form the back contact. Voltage bias was applied to specific devices by contacting with micromanipulator probes appropriate source/drain pads. Voltages up to 40 V<sub>rms</sub> at 10 MHz were applied using a function generator (Agilent 33120A) with a RF power amplifier (EIN - Model 2100L - 50dB).

Earlier studies have indicated this results in Joule heating due to the mobile ions responding to the electric field within the double layer at regions of high electric field above the device. Previous studies have also shown that DNA-in-water solutions have a strong relaxation point in the low MHz regime.(49; 157) This is typically considered to be due to counter-ion movement around the transverse axis of the DNA molecule.(158-160) Using 10 MHz and the movement of ions around the DNA molecule allows heating of a DNA-in-water solution at a much lower frequency than a traditional microwave heater. Hence, even though typically microwaves operate at 2.45 GHz as water has a strong dielectric relaxation point at 17 GHz(161), we are able to use a significantly lower frequency of 10MHz for heating the droplets. A Matlab script was developed to control the function generator output using its embedded GPIB module. In this way, required voltage amplitudes were set and timed creating a voltage ramp for melting curve calibrations.

*Droplet Evaporation*
To quantify droplet evaporation using the AC heating technique compared to a bulk heating technique, the diameter of droplets were measured before and after heating. Volume was calculated assuming a hemispherical shape of the droplet. AC Heating was accomplished by applying $30 \text{ V}_{\text{rms}}$ for 2 minutes. This value corresponds to around 60°C in the core of the droplet. For the bulk heating, a stage heater from Instec Corporation was used to heat the chip and droplets to 60°C. Images were taken before and after heating and the diameter was measured using ImageJ.

*Dehydration/Rehydration of spotted FRET construct*

To confirm this system’s ability to be used as a DNA microarray, we dehydrated FAM-modified ssDNA on devices. These DNA solutions did not contain the Protein Carrier Solution to minimize evaporation. These spots were then rehydrated with Iowa Black Hole Quencher-modified ssDNA. For the match/mismatch test, only one of the spots was rehydrated with the complementary sequence. The other 2 spots were rehydrated with either water or a non-complementary Iowa Black Hole Quencher-modified ssDNA sequence. The droplets were heated using the AC technique and the fluorescence monitored to show an increase in fluorescence from DNA denaturation.

*Droplet evaporation*

To quantify droplet evaporation using the AC heating technique compared to a bulk heating technique, the diameter of droplets were measured before and after heating. Volume was calculated assuming a hemispherical shape of the droplet. AC Heating was accomplished by applying $30 \text{ V}_{\text{rms}}$ for 2 minutes. This value corresponds to around 60°C in the core of the droplet. For the bulk heating, a stage heater from Instec Corporation was used to heat the chip and droplets to 60°C. Images were taken before and after heating and the diameter was measured using ImageJ.
**Image capture and analysis**

To observe the changes in fluorescence, heating of the device took place on a Nikon Eclipse FN-1 fluorescence microscope stage. A B-2E/C FITC filter was used for monitoring the change in the fluorescein fluorescence. As the voltage was swept from 0-40V\textsubscript{rms} at 2.4V\textsubscript{rms} steps for 12 seconds each, a video was taken using NIS-Elements software controlling a Nikon DS-Ri1 camera. These videos were then imported as a stack into NIH’s software ImageJ. Each droplet’s area was selected using oval selection. The mean grey value of the area selected through the entire stack was then measured. This provides a quantitative measurement of each individual droplet’s fluorescence. This raw fluorescence was normalized and then plotted along with its derivative to provide the melting voltage for each droplet.

**Results and Discussion**

**Control of Droplet Evaporation**

The control of droplet evaporation, even at high temperature, is related to the spatial heating profile within the droplet. Simulations show that the unique heating configuration allow for highly localized and well-controlled AC heating above the device at the core of the droplet (Supplementary Figure A.1). Examination of the droplet’s thermal profile shows the temperature at its perimeter has returned to near room temperature. This forms a room temperature encapsulating shell of fluid around the droplet’s heated core, which helps minimize evaporation. By comparison, extreme evaporation of droplets in bulk heating experiments compared to AC heating, provides further justification for use of this localized heating technique for sub-nanoliter droplet-in-air heating (Supplementary Figure A.2).

**Single Droplet Heating**
To demonstrate the feasibility of this methodology, we first focused on temperature-mediated DNA denaturation in individual droplets. A droplet of solution with double stranded DNA (dsDNA) was placed onto a device using microinjection (Fig. 1B and Table 1). For this assay, the 5’ end of the DNA strand and the 3’ end of a complementary DNA strand were modified with fluorescein (FAM) and a black hole quencher (BHQ), respectively(162). The double-stranded conformation of the DNA sequences results in energy transfer between the FAM and the BHQ, producing a low level of observed fluorescence from the FAM molecule. When the dsDNA denatures, the fluorophores separate, FRET efficiency decreases, resulting in an increase in observed fluorescence from the FAM molecule. By modulating the applied voltage, we can control the temperature profile within the droplet (Supplementary Fig. A1). Once a threshold voltage is exceeded, one expects that the dsDNA FRET construct will denature and observed fluorescence will increase (Figure 3.1C).

The heating technique was demonstrated using 3 separate FRET constructs on a single device across multiple chips, as shown in Figure 3.2C & D and Supplementary Figure A.3. Similar to the data from a commercial system shown in the Figure 3.2A & B, the on-chip fluorescence data also shows a sigmoidal curve. The peak of the sigmoidal curve’s first derivative gives the melting temperature of the dsDNA FRET construct. In our system, the peak of the derivative provides a melting voltage, which can be correlated to the known melting temperature of the dsDNA molecule. Multiple tests provided repeatable melting voltages for the FRET constructs (Supplementary Table A.5). Replacement of the FRET constructs with non-modified dsDNA and an observed decrease in fluorescence associated with SYBR Green intercalation corroborated this data (Supplementary Figure A.4, and Supplementary Table A.6). Extracting the melting voltage for the 3 different FRET constructs establishes a calibration curve for melting voltage vs. melting temperature.
Figure 3.2 Single Droplet Melting Curves: (A) A melting curve from commercial real-time PCR machine shows an increase in fluorescence as the FRET construct denatures. (B) Derivative of (A), the peak of which gives the melting temperature of the FRET construct shown in Table 1. (C) On-chip fluorescence data through a voltage sweep from 0-40V\textsubscript{rms}. (D) Derivative of plot (c) showing the melting voltage of the constructs. Averages and standard deviations across multiple chips are shown in Supplementary Table 1. (E) Simulation vs. experimental results for temperature-voltage calibration curve. Different fits are shown with R\textsuperscript{2}-values of linear: 0.854, cubic: 0.586, and theory’s fit: 0.935. (F) Simulation of the time it takes for the temperature to stabilize within the droplet.

Mapping Voltage to Temperature for AC Heating

To understand the physical basis of the calibration curve shown in Figure 3.2E, we solved coupled electrical and thermal equations self-consistently through detailed numerical simulations of the device that includes both the transistor as
well as the droplet (Supplementary Figure A.5). Details of the model are explained in the supporting information, and the numerical parameters used are tabulated in Supplementary Table A.1 and Supplementary Table A.2. Figure 3.2E shows that the theoretical model anticipates, with no fitting parameters, the temperature rise within the droplet well with an R² value of 0.935. Three observations related to heating are easily explained: (a) temperature scales roughly as the square of applied bias, (i.e. $T \sim V_{rms}^2$), (b) despite the inevitable variation of droplet size, the temperature can be set with excellent precision, and (c) steady-state temperature is obtained within milliseconds of the onset of AC voltage (Figure 3.2F). To explain the first observation, recall that the maximum temperature of the droplet, $T_{max}$, is related to the power-dissipated within the droplet approximately as $T_{max} - T_o \approx P x R_{net}$, where $R_{net}$ is the net thermal resistance offered to change temperature, $P$ the power generation due to dielectric heating, and $T_o$ the temperature of the surroundings. Since, the field ($E$) in the device is proportional to voltage ($V$), power scales as, $P = \frac{1}{2} \sigma E^2 \sim V^2$ (Eq. 10, Supplementary Table A.3), where $\sigma$ is the electrical conductivity of the dielectric medium (buffer solution/oxide). Therefore, temperature follows the scaling relationship, $T_{max} - T_o \sim V^2$. Second, to understand the size-independent temperature control, note that heat loss can occur through either the substrate stack or through the droplet. Neglecting the thin bottom oxide layer thickness (0.145μm), the ratio of thermal resistance offered by these two processes can be related to the thermal conductivity of the buffer solution within the droplet ($\kappa_w$) and the substrate region ($\kappa_{Si}$), as, $\frac{R_S}{R_w} \sim \frac{\kappa_w}{\kappa_{Si}}$, where $R_S$ is the thermal resistance of the substrate region and $R_w$ is the thermal resistance of the buffer solution. Since, $\kappa_w << \kappa_{Si}$ (Supplementary Table A.4), the substrate region offers a high conduction path for temperature loss to surroundings. Therefore, $T_{max} - T_o = P(R_S || R_w) \approx PR_{Si}$, where $R_S || R_w$ represents the parallel combination of resistances due to the two regions. Hence, the maximum temperature attained is
mainly determined by the thermal resistance offered by the substrate region \(R_{\text{s}}\) and the temperature of the droplet can be set regardless of the inevitable variation in the droplet size. This is corroborated by experimental results that show droplets with varying diameters show the same heating characteristics (Supplementary Figure A.6). Since, the heat source is localized due to fringing fields, uniformity in the temperature profile inside the droplet increases with decreases in the droplet size (Supplementary Figure A.7). Finally, a time transient analysis of heat conduction (Eq. 9, Supplementary Table A.3) shows that, the temperature saturates quickly to the steady-state value (Figure 3.2F) and hence any measurement done after \(t \sim 10\) ms is stable. Recently, Issadore et al., have reported a similar saturation time due to dielectric heating of water(153).

Application 1: Parallel Melting Curves

The calibration curve, shown in Figure 3.2E, can now be used to achieve specific temperature points required for a variety of biological assays, such as lysing or nucleic acid amplification. For example, to further demonstrate the system’s capabilities, we performed a parallel nucleic acid denaturation study. We shorted the source contact of multiple heating elements and placed individual droplets on each device (Figure 3.3A and Fig 3.1D). Utilizing different dsDNA FRET constructs with varying melting temperatures on linked devices allows us to run parallel melting curves on-chip (Figure 3.3). In this experiment, a single voltage sweep interrogates 3 different FRET constructs. Figure 3.3A shows the progression of increased fluorescence from each droplet as voltage increases. Figure 3.3B & C provide the measured raw fluorescence and derivative of the raw fluorescence vs. voltage for each droplet in the first experiment.
Figure 3.3 Parallel Droplet Heating of Multiple Constructs: (A) A sequence of images showing the process of heating of linked devices for plots (B) and (C). Each droplet contains a unique FRET construct with a different melting temperature (50, 61 and 80°C). (B) A plot of the raw fluorescence data from the droplets during the voltage sweep. (C) The derivative of (b) provides the melting voltage for each of the constructs. Supplementary Table A.3 provides averages and standard deviations for melting curves performed on multiple devices and chips. (D) and (E) provides a second example of linked device heating. In this example, it is possible to discern between two fully complementary strands and a heteroduplex, which contains a single base mismatch. Supplementary Table B.4 provides average and standard deviations for the melting voltage across multiple chips.
To confirm heating uniformity across linked devices (sharing a common source electrode), a single FRET construct was shown to have the same melting voltage across 5 linked devices (Supplementary Figure A.8). This approach provides a simple method of running multiple synchronous DNA melting curves on chip and, by extracting melting voltages of different FRET constructs, a means of quickly developing a calibration curve for each chip in a single experiment or across multiple chips (Supplementary Table A.7).

Application 2: Single Base Mismatch Detection

The ability to distinguish shifts in melting temperature associated with single base mismatches can be important in medical diagnostics and genetic applications. A single base mismatch results in a decrease in the overall free energy of the double stranded complex, which decreases melting temperature (Supplementary Figure A.9). Figure 3.3D & E demonstrate a decrease in melting voltage for a heteroduplex of DNA consisting of a single strand from DNA #4 and the opposing single strand from DNA #5. Wider variation occurred across multiple chips, but the heteroduplex consistently showed a lower melting voltage (Supplementary Table A.8). Heteroduplexes arise from heterozygous PCR amplifications, which are commonly used to determine donor compatibility for organ transplants(163). This system demonstrates the ability to distinguish a single base mismatch using a DNA melting curve within sub-nanoliter droplets and could be used to identify a non-compatible donor pair.
Figure 3.4 Probe DNA Dehydration with Target DNA Rehydration: (A) An example of the process flow is presented. Part A(i) shows the devices prior to dehydration of the probe ssDNA. After spotting of the probe ssDNA (part A(ii)), the DNA in solution is allowed to dehydrate leaving behind residual salts and DNA. Part A(iii) shows a fluorescent image of the dried ssDNA spot. The fluorescence intensity is high without the presence of the FRET quencher. Part A(iv) shows the rehydration of devices 1, 2, and 3. The initial fluorescence before denaturation is show in A(v). The fluorescence intensity is lower than A(iii) due to the introduction of the FRET quencher and the DNA hybridization. (B) and (C) A melting curve of 3 spots that have been rehydrated with a complementary target sequence. The increase in fluorescence shows a distinct, single peak (E) This implies that the DNA has hybridized properly without unwanted heterodimer or self-dimer formation. (D) and (E) A test for specificity in the process. Spots of a dried probe sequence were rehydrated with a complementary sequence, a non-complementary sequence or water. A distinct peak in the derivate in (E) implies a matching sequence.
Application 3: DNA Microarray Technique

We also demonstrate the capability of this system to act as a DNA microarray where each pixel also includes a miniaturized heater. In traditional DNA arrays, probe DNA is spotted on the device. The sample target DNA is then modified with a fluorophore, such as cy-3, and incubated on the probe DNA. The cy-3 target DNA hybridizes to a specific probe sequence, while non-specifically bound DNA is washed away. The resulting cy-3 fluorescence intensity of the spot can be correlated to the amount of target DNA in the original solution (164). This system requires strict control of buffers and hybridization/washing temperatures to minimize non-specific binding associated with false positives. In our version of the system, we utilize denaturation of the DNA complex to determine whether the strands are complementary, a design similar to the dynamic allele specific hybridization (DASH) assay (165). However, our system compartmentalizes melting curves into individual reaction volumes, easily allowing for large-scale parallel analyses.

To demonstrate this technique, we dried a solution of probe single stranded DNA (ssDNA) on the chip’s surface, as shown in Figure 3.4A and Supplementary Figure A.10A. We then rehydrated the dried probe DNA with the single stranded probe DNA target suspended in low evaporation solvent. Similar to standard DNA duplexing techniques, the newly rehydrated probe-target droplet was heated and cooled once to ensure proper DNA hybridization (Supplementary Figure A.10 B & C). This initial heating curve showed dual-peaks in the derivative, which can be attributed to improper hybridization that occurs when DNA is duplexed at room temperature. In order to confirm that the dual-peak nature of the initial heating/cooling step was due to improper hybridization, we dehydrated a fully duplexed FRET construct on the chip surface and then rehydrated it. As shown in Supplementary Figure A.11, the initial melting curve for this complex showed the single peak of a fully hybridized DNA
complex. Immediately after the initial heating/cooling step, a second melting
curve was run which now showed a single peak in the derivative. This affirms
that the probe and target were fully hybridized and complementary (Figure 3.4B
& C). To confirm the specificity of this technique, we dehydrated a probe ssDNA
strand on 3 separate devices. We then rehydrated one device with a
complementary ssDNA, one device with a non-complementary sequence, and one
device with water as a control. Figure 3.4D & E show that only the
complementary matching sequence show an increase in fluorescence. The
mismatch and water show no increase in fluorescence from DNA denaturation.
This methodology is compatible with current DNA microarray technologies and,
in addition, promises to extend the capabilities of current DNA microarrays and
DASH platform by including a FRET fluorophore, like cy-5, in the spotted probe
DNA; incorporating a heating element under each spot on the array; and utilizing
droplets-in-air for individual reaction compartments.

Conclusions

Integration of various laboratory functions onto microchips has been intensely
studied for many years. Lab-on-a-chip technologies are attractive since they
require fewer reagents, have higher detection limits, allow for parallel analyses,
and can have a smaller foot-print. Further advances of these technologies require
the ability to integrate additional elements, such as the miniaturized heating
element described here, and the ability to integrate heating elements in a
massively parallel format compatible with silicon technology(166). Notably, our
miniaturized heater could also function as dual heater/sensor elements, as these
SOI nanowire or nanoribbon structures have been used to detect DNA, proteins,
pH and pyrophosphates(89; 167-170).

In summary, by utilizing micro-fabrication techniques and incorporating
the novel design of transistor-based heating with individual reaction volumes,
‘lab-on-a-chip’ technologies can be scaled down to ‘lab-on-a–transistor’
technologies that exist as sensor/heater hybrids for point-of-care diagnostics. We elucidate a technique to heat sub-nanoliter droplets-in-air for visualization of DNA denaturation with resolution down to single base mismatches with application to current DNA microarray technologies. This methodology can be extended to a variety of other high-throughput screening applications such as high-speed PCR, single cell lysis, single molecule enzymology, and interrogation of ligand-receptor interactions in protein melting studies.
CHAPTER 4

SINGLE CELL MANIPULATION AND ULTRA-LOCALIZED CELL LYSIS USING SILICON NANOWIRES

Abstract

Analysis of cell-to-cell variation can further the understanding of intracellular processes and the role of individual cell function within a larger cell population. The ability to precisely lyse single cells can be used to release cellular components to resolve cellular heterogeneity that might be obscured when whole populations are examined. We have previously demonstrated localized fringing electric fields at a transistor’s surface using an AC voltage mediated strategy. Here, we report through experiments and simulations that the electric fields generated directly above the device enable electroporation at applied voltages of less than 1 V. In this study, HT-29 cancer cells were positioned on top of transistors by manipulating magnetic beads using external magnetic fields. Ultra-rapid cell lysis was subsequently performed by applying 600-900 mV_{pp} at 10 MHz for as little as 2 ms across the transistor channel and the bulk substrate. We show that the fringing electric field at the device surface disrupts the cell membrane, leading to lysis from irreversible electroporation. This methodology allows rapid and simple single cell lysis and analysis with potential applications in medical diagnostics, proteome analysis and developmental biology studies.

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Introduction

Analysis of cellular content, such as proteins and nucleic acid molecules typically requires a-priori cell lysis by rupture of the cell membrane. This can be achieved by chemical,(171) electrical,(172; 173) enzymatic,(174) mechanical(16; 175-177) or thermal(22; 178) means. Each technique presents unique advantages and disadvantages that must be considered to ensure compatibility with downstream analysis.(179) For example, conventional thermal lysis is a bulk and non-specific process that leads to breakdown of cell membranes and cell compartments, and can cause denaturation of biomolecules such as proteins and nucleic acids. This can lead to degradation of analytes such as proteins, thus hindering integration of thermal cell lysis with analysis of cellular content.(180)

An alternative lysis methodology is cellular electroporation, which has been discussed thoroughly in literature.(181) In electroporation procedures, an applied electric field creates a potential across the cell membrane, known as the transmembrane potential (TMP). Once this potential exceeds a certain threshold, ~0.2-1.0V, pores will form in the cellular membrane.(19) Variations in the size and number of pores formed depends on the duration and strength of the applied electric field as well as the cell type, developmental stage, medium and cellular dimensions. Depending on their size and number, these pores will either reseal (termed reversible electroporation) or they will remain open resulting in irreversible electroporation. In irreversible electroporation, cellular contents are released and the cell dies. This technique can allow for molecular analysis of released contents with minimal induced damage. Since the first single cell electroporation in 1998,(182) several methods for single cell electroporation have been developed(183) utilizing platforms like microfabricated chips(184; 185) and vertical nanopillars.(186)

We have previously demonstrated localized fringing electric fields at a transistor’s surface using an AC voltage mediated strategy.(3; 4; 187) In the current work, we show through experiments and simulations that the electric
fields generated directly above the device enable electroporation at applied voltages of less than 1 V. In this approach, the electric field is highly localized at the nanometre scale above the device surface, which enables specific, single cell lysis.

In order to electroporate single cells, cells must be individually targeted and positioned. A variety of methods have been used to controllably position cells including patterning,(188) fluidic traps and wells,(189) optical traps,(190) dielectrophoresis,(22) and others.(191) The method in this study incorporates a label-free and trap-less technique where magnetic beads are rolled to push and pull untethered or non-specifically–bound cells into a desired position.(192-194) This technique is attractive, as it does not require transparent substrates needed for optical tweezers or high voltages on chip and strict control of media conductivity required for dielectrophoresis.

In this paper, we demonstrate integration of magnetic manipulation techniques with a robust cell lysis technique. By using field effect transistors to apply an electric field to cells, we enable a gentle and specific cell lysis with potential for field effect sensing of released cellular components. This would enhance the usability and portability of lab-on-a-chip devices by minimizing loss of biological molecules.(195) We envision this system being used in single cell analysis studies that focus on cell-to-cell variation within a population.

Materials and Methods

Chip fabrication

Fabrication details of the silicon field effect devices using a Silicon on Insulator (SOI) wafer have been described elsewhere.(196) (see Figure 4.1 for device architecture) Silicon nanowires (SiNW) and silicon nanoribbons (SiNR) were used. The SiNWs had a width of 50-100 nm and a thickness of 30 nm whereas the SiNRs had a thickness of 50 nm, but a width of 2 μm.
Surface preparation

To prevent premature cellular adhesion, the surfaces were passivated using an organosilane monolayer. The process consisted of an oxygen plasma treatment for 2 min, then, 50μl of 5% 3-aminopropyltrimethoxysilane, APDMS (Gelest Inc.) in toluene is pipetted onto the reactive surface and incubated for 15 min. The surface is rinsed with toluene, methanol, DI water and wiped with a microfiber.
swab. The device was then treated with trypsin-EDTA (0.25 % trypsin-0.53 mM EDTA in HBSS without calcium and magnesium, ATCC) before cell addition to further reduce cell adhesion. Excess trypsin-EDTA was rinsed off with DI water prior to device usage.

**HT-29 cell culture and preparation**

HT-29 cells (human colon carcinoma, ATCC # HTB-38™) were used throughout the study and prepared using standard cell culture conditions with DMEM (Invitrogen corp, Carlsbad, CA), supplemented with 10% FBS (Sigma Aldrich, St. Louis, MO) and 1% penicillin-streptomycin.

**Cell positioning with magnetic beads**

Cell positioning on devices utilized the programmability of magnetic field routines for magnetic manipulation of 7.9 μm COOH-modified COMPEL Magnetic Microspheres from Bangs Laboratories, Inc. ([Figure 4.2(a) and (b)]). Details on this setup and its operation can be found in prior work.(192-194) To ensure stable measurements, the positioned cells are allowed to adhere to the device surface for 30 min prior to lysis.

**Cell lysis**

The setup used was described previously, but at higher voltages.(3; 4; 187) In this study, voltages at 10 MHz and up to 900 mVpp were applied between the shorted source-drain and the back gate of a transistor, shown in a schematic representation ([Figure 1 (a) & (b)]).
A live-dead assay for monitoring cell membrane integrity was adapted from Privorotskaya et al. (178) Propidium iodide (PI), a membrane impermeable dye, and DiOC$_6$(3), a membrane dye, from Sigma Aldrich were used. Upon breakdown of the cell membrane, PI enters the cell and intercalates with the cellular DNA. An increase in PI fluorescence was used to determine cell lysis. Lysed and intact cells were also fixed using a similar protocol to Privorotskaya et al. (178)

**Results & Discussion**

**Cell positioning characterization**

The two variable parameters of the magnetic positioning system,
frequency (Hz) and strength (Gauss), of the rotating magnetic field were characterized for the magnetic beads to optimize speed and control of the movement of cells. With optimization of the surface preparation as well as the applied field strength and frequency, magnetic beads could be reliably used to position cells. As shown in Figure 4.2(c) and (d), cells of various types can be reliably positioned directly above the transistors for later lysis.

Cell electroporation characterization

The membrane dye, DiOC₆(3), and the membrane-impermeable dye, PI, were added to the solution of cell media containing HT-29 cells and magnetic beads. Bright field and fluorescent images of cells positioned on devices were taken before and after an application of 10 MHz pulse of 1200 mV_{pp} for 1 min and during 60 min incubations (Figure 4.3(a), (b)). The time course of fluorescence (Figure 4.3(b)) shows PI (red) fluorescence increasing after voltage application, which implies degradation of the cellular membrane. SEM images of an intact cell and a lysed cell were also taken (Figure 3(c) and (d)). The SEM of the lysed cell shows significant breakdown of the cell membrane from the applied signal. Supplementary Figure B.1 shows the highly localized nature of this method. Only a cell located directly on top of the transistor is lysed, while cells immediately adjacent are unharmed. Experiments to determine the lysis threshold were also carried out. Voltages of 300, 600, and 900 mV_{pp} were applied. 0% (n=10) of cells at 300 mV_{pp} for 1 min were lysed, 83% (n=12) of cells at 600 mV_{pp} for as little as 2 ms were lysed, and 100% (n=5) were lysed at 900 mV_{pp} for 1 s. The threshold for lysis is likely between 300 and 600 mV_{pp}.  

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Figure 4.3 Single Cell Electroporation: (a) An MCF-7 cell on the far left nanoribbon is lysed via an applied signal of 600 mVpp, 10 MHz for 2ms. Two live cells on or near the second nanoribbon from the left as well as one already dead cell on the far right are shown as reference. Images (i-iii) show DiOC₆(3) (green) and PI (red) fluorescence signals from these cells. (i) Shows the cell before applied voltage. (ii) Shows immediately after the signal was applied. After 20 minutes (iii), PI fluorescence in the cell has increased implying cell death. The control cell on the nanowire to the right shows no PI increase over the duration of the experiment. (b) Shows the time course of PI fluorescence increase for an intact cell and a lysed cell. (c) An SEM of an intact cell. (d) An HT-29 cell that has been lysed by applied signal to a nanowire is shown. Scales bars are (a) 100 μm, (c) 5 μm, (d) 10 μm
Lysis mechanism

The previous chapter highlighted the ability to localize electric fields directly about the gate region of a transistor for heating applications. In this work, the applied voltage is significantly lower and should not induce significant heating. Lysis of the cells is believed to be due to local electroporation of the cell membrane. To confirm that the cell lysis was due primarily to electroporation and not thermal lysis, the temperature profile above the device was interrogated to determine whether the voltages used for cell lysis would generate any joule heating or AC heating. We used a dsDNA molecule, modified with a FRET acceptor on one strand and donor on the other strand, which was added to pure cell culture media. This dsDNA FRET construct was designed to denature at 70°C and upon denaturation, the observed fluorescence would increase due to separation of the FRET donor and acceptor pair. The voltage was increased at 2.4 V\text{pp} increments every 10 seconds up to 100 V\text{pp}. Measurement of three zones extending away from the device showed no observed fluorescence increase from heating at the low voltages used for cell lysis (Supplementary Figure B.2). Fluorescence from DNA denaturation only increased above 64 V\text{pp}, which corresponds to values reported in previous studies using this technique.\(^3\)

Current-Voltage characterization was also performed before and after electroporation pulse application to ensure the applied signal did not damage the device’s electrical characteristics (see Supplementary Figure B.3). The results show that the threshold voltage and saturation current are only slightly altered; however, the device retained transistor characteristics and had low current leakage post-lysis.

Conclusions

Here we present a novel method for lysis of single cells positioned directly above a field effect transistor. This technique has the potential to enable many applications that require specific, localized cell lysis on a biosensing platform.
We describe a simple method for releasing biological components from the cell, which reduces the impact on molecule stability from thermal, chemical, or mechanical degradation. Label-free and trap-less magnetic manipulation of cells especially overcomes the need for transparent substrates in optical tweezer techniques; the need for microfluidic trapping techniques; and the need for high voltages and low media conductivity in dielectrophoresis-based trapping of cells. This methodology can be applied to a wide variety of biological studies that focus on single cell vs. cell population analysis.
CHAPTER 5

ELECTRICAL DETECTION OF NUCLEIC ACID AMPLIFICATION USING SILICON FIELD EFFECT TRANSISTORS

Abstract

Electrically-based detection of nucleic acid amplification through pH changes associated with nucleotide addition enables miniaturization, greater portability of testing apparatus, and reduced costs. However, current ion-sensitive field effect transistor methods for sensing nucleic acid amplification rely on establishing the fluid gate potential with a bulky, difficult to fabricate reference electrode that limits the potential for massively parallel reaction detection. Here we demonstrate a novel method of utilizing a microfabricated solid-state electrode paired with a pH-insensitive REFET for detection of pH changes from loop mediated isothermal amplification. The 0.18µm, SOI, foundry process, QRE-REFET sensor system utilizes a solid-state, quasi-reference electrode (QRE) to establish the pH-sensitive fluid gate potential and a passivating membrane on the ISFET to enable pH detection of LAMP. This technique is highly amendable to commercial scale-up, reduces the packaging and fabrication requirements for ISFET pH detection, and enables massively parallel droplet interrogation for applications such as digital PCR.

This work will be submitted for publication.
Introduction

Since the invention of Polymerase Chain Reaction (PCR)-based amplification of nucleic acids by Kary Mullis in 1983(1), researchers have spent significant efforts to improve the sensitivity and selectivity of PCR assay and have dramatically enhanced its application. PCR is now an integral tool of modern biotechnology processes and biological identification. Due to the growing demands of on-site diagnosis in medicine, realization of point-of-care polymerase chain reaction (PCR) strategies has garnered much attention.(2) Beyond traditional PCR, digital PCR for absolute quantification, increased robustness, and higher sensitivity is highly desirable. Such a strategy would ideally be portable, simple, rapid, have a low cost per test, high accuracy and reproducibility, require minimal sample volumes and concentrations, and be capable of multiplexed interrogation for many relevant species. Of these criteria, the three most important barriers impeding the development of a point-of-care PCR or digital PCR test are: (i) the time for performing the thermal cycles, (ii) high cost and complexity of required equipment, and (iii) the lack of a truly miniaturized, portable solution.

Much effort, both academic and commercial, has focused on addressing these barriers, though typically only on one at a time. In terms of time, Xxpress(197) offers a qPCR thermal cycler that can achieve 40 cycles of qPCR in less than 10 minutes (with a ramp rate of ~10 °C per second), but with a bulky and expensive tabletop system without automation. RainDance(198) and BioRad(199) offer extremely high throughput digital PCR machines utilizing millions of droplets to enable quantification of the initial copy number of the target nucleic acids, but the process takes well over an hour and requires a large table-top system from droplet generation and interrogation.

Beyond thermocycling times, the other major source of cost and complexity comes from the use of fluorescence-based strategies for detection. A number of factors make optical detection of nucleic acids in miniaturized systems
challenging to accomplish. From a reagent perspective, the fluorescence increases with concentration of the fluorophores, but with higher concentrations come much higher costs. Second, optical detection requires PCR product markers such as SYBR Green or Taqman probes, which can induce inhibitory effects on PCR for SYBR Green and increase the per assay cost of each PCR reaction.(200) From an equipment standpoint, optical techniques require bulky, expensive detection equipment. Typically, both a light source to excite the fluorophore and a detector to receive the incoming photons are required. Many efforts have attempted to miniaturize these components and bring costs down, but few examples of portable inexpensive nucleic acid amplification exist. Optical PCR systems remain relatively expensive and cumbersome.

Electrical detection, on the other hand, avoids most of these disadvantages by eliminating the need for fluorophores and optical detection equipment entirely. To date, systems utilizing ion sensitive field effect transistors(ISFETs)(5; 201), MOS-capacitors(202), and interdigitated electrodes(49; 203) have demonstrated successful detection of nucleic acid amplification. Of these methods, FET arrays offer benefits of fabrication scalability and row-column addressing, which make them an attractive option for parallel detection assays. Sophisticated semiconductor fabrication foundries offered by companies such as Taiwan Semiconductor Manufacturing Company (TSMC) can manufacture devices with high yield, ideal and highly repeatable device characteristics, and huge amenability for scale-up.

Recent years have seen advancements in use of ISFETs for detection of small molecules and biological reaction by-products, such as hydrogen ions. Although first introduced in the early 1990s(204), DNA Electronics has recently come to the forefront of developing and commercializing this technique. In 2001, Toumazou et al. demonstrated detection of nucleotide incorporation events through detection of hydrogen ions in up to 5 simultaneous reactions.(5; 201; 205)
This work has extended to sequencing applications and is currently being developed by Ion Torrent by Life Technologies.

However, to date, commercial ISFET applications have required the use of a macroscale Ag/AgCl reference electrode to apply the necessary fluid gate biasing for FET operation. Although Rothberg et al. demonstrated a FET based DNA sequencing system utilizing massively parallel nucleotide incorporation detection with millions of reaction wells using a single reference electrode(166), this assay is limited to reactions that occur in pulses through sequential addition of nucleotides. Nucleic acid amplification techniques, such as PCR or loop mediated isothermal amplification (LAMP), require much longer time courses during which diffusion of hydrogen ions away from a non-compartmentalized reaction would limit parallel detection. Use of an on-chip microfabricated electrode with encapsulation of each reaction volume can enable the parallel detection of many individual reactions, completely eliminating crosstalk between reaction chambers that are physically isolated from one another.

Here we detail the steps required to realize a methodology towards this goal. Due to the pH-sensitivity of solid-state electrodes(206; 207), a REFET design was used. A pH-passivating membrane on a standard ISFET monitored the pH response of the electrode. Previous techniques have demonstrated the use of pH-insensitive layers over ISFETs, such as silanes, buffered hydrogels, parylene, and polyACE.(208) However, polyvinyl chloride (PVC) offers an attractive alternative due to its previously demonstrated simple fabrication process, robust pH insensitivity, and known compatibility with PCR with added BSA.(209-212) Together, the combined system of a pH-sensitive electrode and a pH-insensitive REFET offers an opportunity for on-chip electrical detection of biological reactions, such as loop-mediated isothermal amplification, targeting a variety of pathogens. This strategy enables isolated and autonomous reaction chambers that can detect many separate reactions simultaneously.
Materials and methods

Device Fabrication

Taiwan Semiconductor Manufacturing Corporation (Hsinchu, Taiwan) used a standard foundry process to manufacture 0.18 μm node extended gate ion-sensitive field effect transistors as seen in Figure 5.1(b). Metal 1 was deposited to make contact to the source, drain, and to serve as the extended gate area connected to the polygate regions. Next, an interlayer dielectric (ILD1) was deposited. Vias in ILD1 were etched to form 40μm by 40μm openings to the extended gate structure and exterior source/drain electrode pads for probing. A layer of hafnium oxide was then deposited over the entire chip to form the oxide sensing membrane on the extended gate structure. At UIUC, 200Å titanium/800Å platinum electrodes that were used as the fluid gate contact were patterned on top of the hafnium oxide dielectric layer using standard photolithography and evaporation steps (see Figure 5.1(c)). For the on-chip REFET, the chip was first silanized using HMDS for 1 hour at 140°C. The chip was then cleaned of any excess HMDS using consecutive rinses of acetone, methanol, DI water, and isopropanol. A mixture of 60% PVC, 40% dinonylphthalate (DNP), suspended in 3mL of tetrahydrofuran (THF) was used for passivating the REFET.(210) 1uL of PVC/DNP suspended in THF was then spotted on the half the devices and baked at 80°C for 1 hour (see Figure 5.1(a)). Before chips were measured, a final, 1 minute oxygen plasma step was used to standardize the chip surface and prime the platinum surface.
Figure 5.1 Device schematic and characterization: (a) A generic device schematic is shown. Two fluid gate methods are shown. A pH-insensitive leak-free electrode and a pH-sensitive on-chip platinum electrode. PVC covers one of the ISFETs, rendering it insensitive to pH changes. The expanded view depicts the pH-sensitive nature of both the ISFET gate oxide and the on-chip platinum electrode. A generic nucleotide incorporation event results in generation of a hydrogen ion which alters the pH of the solution and the pH-sensitive fluid gate potential. (b) An extended gate ISFET is shown. The sensing region consists of a layer of hafnium oxide on a metal gate. (c) A 1uL PVC is spotted on a portion of the devices to render them pH insensitive, but still functional. (d) A real-time pH response curve of two untreated ISFETs is shown. Four additions of NaOH, followed by four additions of HCl is shown. The response closely matches the Nerstian response for ISFET gate dielectrics with an average pH response of 57mV/pH and R^2 linearity of .995. (e) Quantification of the HCl addition steps for multiple devices is shown. (f) The real-time response curve for an untreated (blue) and a PVC-treated (red) with a leak-free reference electrode is shown. The PVC-treated device shows minimal pH response over the duration of the test.
Experimental Setup for FET Measurements

Current measurements and applied biases were controlled using a Keithley 4200 semiconductor characterization system. Contact to the chip electrode pads was accomplished using micromanipulators from The Micromanipulator Company. For fluid gate measurements, a 400µL, 10:1 PDMS well was placed on the chip. For reference measurements, fluid gate biases were applied with a leak-free Ag/AgCl reference electrode (Warner Instruments). For quasi-reference measurements, a micromanipulator was used to contact the on-chip platinum electrode. For pH titration experiments, 2mM Tris buffer at pH 7.06 was used. Subsequent additions of 0.1M NaOH or HCl were used to vary the pH. Baseline measurements were taken with the leak-free electrode to characterize the TSMC chips with and without PVC (see Figure 5.1 (d-f)). Separate calibration experiments using an In-Lab Ultra Micro pH Probe from Mettler Toledo were completed to quantify the pH response from a given NaOH or HCl addition event (see Supplementary Figure C.1).

FET Testing and Surface Potential Measurements

Initial $I_D-V_G$ curves measuring drain current while sweeping the fluid gate were extracted with first the reference electrode and then by the quasi-reference platinum electrode. For pH titrations, current vs. time measurements were taken with the FET set in the linear regime. In order to standardize measurements for different devices, the measured current was compared to the baseline $I_D-V_G$ curve to extract the extended gate surface potential (see Figure 5.1(d-f) and Figure 5.2).

In order to quantify the pH sensitivity of the platinum QRE, the surface potential of the QRE vs. the leak-free reference electrode was measured using the Keithley 4200. The open circuit potential between the pH-insensitive RE and the QRE was measured with two separate Source Measuring Units (SMUs). Without
a final oxygen plasma treatment step, the pH response was unstable as is shown in Supplementary Figure C.2.

Figure 5.2 FET Response with Platinum Fluid Gate: (a) A real-time pH response curve with 4 subsequent NaOH additions. The PVC and non-PVC response curves are shown. The non-PVC ISFET shows almost zero response when operated by the platinum electrode. (b) The pH response is quantified for many devices across multiple chips (n=XXX). The PVC REFET sensor shows an apparent opposite trend than expected with hydrogen addition. This is due to the charge at the platinum electrode being mirrored into the REFET response. (c) A real-time pH response curve with four subsequent NaOH additions is shown. (d) The pH response is quantified across multiple devices. The non-PVC ISFET shows minimal pH response compared to the PVC-REFET.
Initial proof-of-concept LAMP experiments were completed with the EIKEN kit for *E. coli* O157:H7. Further experiments that required modification of the LAMP solution were based on LAMP formulations from New England BioLabs’ recommendations for LAMP. Primers used for the various non-O157 shiga toxin-producing *E. coli* are found here.(213) A 25uL reaction mix consisted of 0.05X-2X Isothermal amplification buffer from New England BioLabs, 800mM Betaine from Sigma-Aldrich, 50mM KCl, 1.9uM FIP and BIP primers, 0.24uM F3 and B3, 0.96uM Loop-F and Loop-B primers, 1X EvaGreen from Biotium, 6 units of Warmstart Bst 2.0 polymerase from New England BioLabs, 1.3mM dNTPs from New England BioLabs, 5mM MgSO$_4$ from Sigma-Aldrich, and template of our targeted *E. coli* O26 template.

Optimization of the reaction centered around three major areas: (1) tris-HCl buffer concentration, (2) starting pH value, and (3) reaction temperature. Electrical detection of LAMP was carried out by first running part of the solution in an Eppendorf RealPlex qPCR system. After completion, the portion of solution that was not amplified was measured on the FET with either the RE or the QRE, followed immediately by the amplified solution. Importantly, to minimize electrostatic discharge effects when exchanging solutions, the micromanipulator tips were lifted off the device pads in between measurements. Electrostatic discharge events have been shown to cause large shifts in the device threshold voltage and should be minimized whenever possible. (214)

**Results and discussion**

**Characterization of TSMC Chip**

Utilizing a semiconductor foundry for fabrication of ISFET offers several distinct advantages. Each chip from TSMC supplied devices with greater than 95% yield. With the fabrication control afforded by the semiconductor foundry, charge
trapped in the dielectric was controlled through anneals and minimally affected device stability. Threshold voltage uniformity across devices was excellent and the standard deviation of the threshold voltage of each individual device over 5 sweeps from cut-off to saturation was on the order of 0.1-1 mV. Assuming a Nernstian pH response, this noise level should enable a pH resolution on the order of 0.005-0.05 pH units. Hafnium oxide, a high-k dielectric that increases the coupling capacitance to the device for a given thickness, has been shown to provide near Nernstian pH response. This was evident in the baseline pH testing shown in Figure 5.1 (e). The pH titration in real-time method over a relatively small pH range (pH 7-8), showed some instability likely due to inaccuracy while pipetting. However, simultaneous measurement of two devices as shown in Figure 5.1 (d) demonstrates sensor response uniformity and stability even when testing across the 5 mm sensing region of the chip.

Characterization of PVC-REFET and Platinum Response

Researchers have explored the use of REFETs for pH sensing applications starting shortly after the first ISFET was introduced in the 1970s.(216) The REFET enables the user to normalize for unexpected changes in electrode potential that can originate from factors not due to pH changes, such as temperature instabilities that can impact ISFET sensor response. Typically, a pH insensitive REFET is paired with an ISFET in a differential setup. Much work focused on a reliable method for properly rendering the REFET pH insensitive. Early work focused on minimizing the number of available hydroxyl groups at the device surface via the introduction of a silane layer that covalently reacted with the dielectric surface and reduced the available hydroxyl groups. This method has seen limited practice due to the difficulty of occupying a high enough percentage of available groups. Bergveld et al. showed a reduction of pH response of the ISFET to form a pH-insensitive REFET required a 99.99% reduction of hydroxyl
groups. As shown by Tarasov et al., this requires a silanization procedure that takes up to seven days in a vacuum oven.

Other early methods, such as ion-blocking layers of photoresist or other polymers like parylene or PVC, have also been demonstrated. These methods primarily rely on a macroscale Ag/AgCl reference electrode for the fluid gate, which is bulky, expensive, and difficult to fabricate. Microfabricated Ag/AgCl electrodes also suffer from potential instability and reduced lifetime when submerged in solutions less than 3M chloride, which is often incompatible with biological applications. Many examples have utilized a solid-state electrode as the fluid gate, but each used a differential signal between the ISFET and a REFET for pH sensing. By elucidating the platinum pH response in tris buffer, as seen in Figure 5.3, this work eliminated the need for a differential signal with an ISFET. By blocking the ISFET’s pH response with spotted PVC, the resulting current trace from the ISFET shows the pH response of the platinum. When using the same surface potential extraction method as the baseline case, the sensor shows the opposite signal to the addition of NaOH or HCl (Figure 5.2). This overall pH sensitive system (~34-36 mV/pH) responds to the change in potential at the platinum fluid electrode and not the gate dielectric surface potential. Each case demonstrated a linear relationship between the pH and the REFET response. When the QRE platinum electrode is used in conjunction with a device without PVC, the surface potential response of the platinum and the gate dielectric mirror each other. This results in a lack of pH sensitivity in the system for this case. In essence, the ISFET can become the reference to the platinum electrode.

Additionally, we have shown that the PVC membrane and solid-state electrode can be used for pH detection in droplets-in-mineral oil (see Supplementary Figure C.3). It was noticed that PVC would delaminate from the chip surface upon submersion in mineral oil. By baking the PVC at 80°C
before submersion, the PVC is able to maintain integrity for over 3 hours at room temperature and over 1 hour at 65°C (Supplementary Figure C.4). This adds the potential for droplet applications, such as digital PCR and digital LAMP, that have gained popularity in recent years. (223)

**Figure 5.3 Platinum Surface Potential Response:** (a) A schematic of the open circuit potential method is shown. The on-chip platinum vs. the leak-free electrode are probed using the Keithley 4200 semiconductor characterization system. The current for both nodes is held at zero and the resulting potential between the two nodes was measured. (b) A real-time plot of four HCl additions is shown. (c) The surface potential from the platinum response is extracted and quantified. The pH response shows high linearity and sensitivity that closely resembles those stated previously.
Loop mediated isothermal amplification (LAMP) was developed in the early 2000s as an isothermal alternative to PCR. (224; 225) LAMP utilizes four distinct primers recognizing six regions of a targeted gene. This method offers high sensitivity (shown in Figure 5.4(a)) and superior specificity to PCR, which enables an inherently non-specific method of amplification detection, such as an intercalating dye or, in this case, pH detection. LAMP provides a yield of >500ug/mL of DNA. PCR, on the other hand, only offers a maximum yield of around 40ug/mL. (225) The level of DNA generation in LAMP results in a higher potential pH change for a given buffer concentration. Theoretically, in standard buffered amplification solution of 20mM tris-HCl, LAMP will yield a pH change of ~0.136 units, whereas PCR will only produced a pH change of ~0.01 units. (See supplementary information for a description of the pH change calculations). As shown in Figure 5.4(b), using the Eiken kit for E. coli O157:H7, the LAMP reaction generates a pH change ranging from -0.15 to -0.20 pH units. This change is consistent across the entire range of starting template concentration since each reaction was allowed to run to completion.

**Optimization of LAMP Reaction Conditions**

The LAMP optimization process with respect to pH changes focused on three major areas: (1) Tris-HCl concentration, (2) starting pH value, and (3) reaction temperature. In a traditional amplification reaction, a highly buffered solution maintains a consistent pH in order to maximize polymerase. For a pH-based amplification reaction, the buffering capacity must be reduced, ideally without hindering polymerase activity. Figure 5.4(c) shows the pH change from LAMP versus a range of Tris-HCl concentrations, revealing the yield and pH change is consistent with expectations from 40mM-8mM. 4mM reduced yield of the LAMP reaction, which slightly diminished the pH change. This behaviour may be explained by the reduction in initial pH associated with diluting the buffer with DI
water. When the starting pH was increased to around pH 8, the yield increased and followed expectations more closely.

Figure 5.4 pH-LAMP Optimization: (a) The detection limit of a commercially available *E. coli* O157:H7 kit is shown. LAMP allows for detection of 10-100 CFU/reaction in less than 30 minutes. (b) The pH-LAMP detection limit is shown. Regardless of starting *E. coli* concentration, the resulting pH change is consistent at around -0.2 pH units. (c) Reducing the Tris-HCl buffer concentration in the reaction mix increases the pH change associated with amplification. The maximum pH change observed in these tests was -1.2 pH units. (d) Decreasing the isothermal amplification buffer concentration also reduced the ionic strength of the solution. This requires the reaction temperature to be reduced to achieve consistent threshold times. Threshold times were consistent down to 8-12mM Tris-HCl before significant increases in threshold time are observed.
Dilution of the Isothermal Amplification Buffer also reduced the ionic strength of the solution. Without replacing the missing salts, the melting temperature of the dsDNA in solution decreases. As shown in Figure 5.4 (d), lower buffered solutions required a lower reaction temperature to see Threshold Times similar to higher buffered solutions. In the case of the 4mM solution, the addition of 50mM KCl increased the ionic strength of the solution and improved the threshold times observed. (data not shown).

End-point Detection of LAMP On-Chip

Toumazou et al. previously demonstrated the potential for pH-based detection of nucleic acid amplification using an ISFET.(5; 201; 205) However, their system requires a reference electrode to establish the fluid gate potential and operate the ISFET. This technique is demonstrated in end-point measurement form in Supplementary Figure C.5. This method advances the potential for portable nucleic acid amplification detection; however, on-chip reference electrodes are still fairly large and difficult to fabricate, minimizing the chance for massively parallel, portable amplification detection. By utilizing a solid-state electrode, patterned with standard photolithography and evaporated onto the chip, we added the potential for massively parallel amplification detection.

In order to demonstrate this potential, we have shown detection of a LAMP reaction using end-point pH measurements with a solid-state electrode and an ISFET passivated with PVC. Macroscale pH measurements with the In-Lab Ultra Micro pH meter showed a pH change of -1.24 units for the complete full reaction. As shown in Figure 5.5 (b) and (c), without the PVC membrane, the positive and negative amplification reactions are indistinguishable (two-tailed p-value = 0.7745). Compared to the negative reaction, the PVC REFET shows strong results that are statistically significant (p-value < .01). The PVC-REFET responds to the change in the electrode potential, which is sensitive to the pH of the solution. Supplementary Figure C.6 shows the stability of the device
threshold voltage with repeated solution exchanges. The LAMP end-point data was statistically significant compared to variations in the threshold voltage associated with solution exchange (p-value < 0.0001).

Conclusions

Here, we present a novel technique for electrical detection of nucleic acid amplification. Utilizing a field effect transistor in conjunction with a solid-state electrode simplifies pH-based detection of biological processes, such as LAMP. This technique has the potential for many applications that require electrical detection of pH changes in droplets. Disciplines that could see significant benefit from this methodology include digital PCR or digital LAMP, as well as assays enabled by electrowetting-on-dielectric droplet manipulations. Researchers could use this to perform high throughput analysis of enzyme activity, detect the presence of a targeted nucleic acid sequence, or detect the presence of a targeted biomolecule through monitoring an enzymatic reaction’s rate and progress through hydrogen generation. This methodology enables droplet interrogation and simplifies macro-scale solution interrogation through the use of common metal patterning techniques and a pH-insensitive REFET.
Figure 5.5 Endpoint Detection of LAMP On-Chip: (a) I-V curves of a PVC-REFET device is shown. The differences between the negative control before and after amplification are not significant. The positive control shows a shift in the positive direction, which is consistent with a decreased pH. The pH change was also measured to be -1.24 units with a commercial meter. (b) Measurements were taken simultaneously with a non-PVC ISFET. The positive and negative amplification solutions show insignificant differences. (c) The change in threshold voltage was quantified for multiple devices. The change for positive amplification was statistically significant when compared to the negative control for the PVC-REFET. The non-PVC ISFET showed no significant changes.
CHAPTER 6

CONCLUSION AND FUTURE OUTLOOK

Through expanding the uses of the field effect transistor platform to include a novel design of heating/cooling, ultra-localized cell lysis, and electrical detection of nucleic acid amplification using an on-chip electrode, this thesis aims to enable the next generation of portable biosensors for primary care and on-site diagnostics. Label-free electrical detection of PCR amplification in a robust and reliable manner to result in point-of-care PCR in the primary care setting would revolutionize molecular diagnostics, and have major impact on all areas of DNA-based diagnostics. The silicon CMOS compatible technologies we have proposed can easily be multiplexed so the ultimate vision of an array of PCR reaction chambers, where the target sample can be interrogated against an array of primers, can be realized. This thesis has added three new functionalities to the transistor platform. 1) Rapid DNA denaturation 2) Localized single cell lysis and 3) Electrical detection of amplification by-products with an on-chip electrode.

As discussed in chapter 2, a major challenge facing researchers is the integration of methodologies into a single portable platform. To date, few strong examples exist where lab-on-a-chip technology has been successfully brought from ‘lab-bench to the bedside’. Here I outline a vision of how each of this thesis’ individual technologies could be brought together into a single platform capable of multiplexed, digital LAMP targeting a variety of pathogens. This future outlook will focus on microfluidic sample partitioning, localized cell lysis, further development of electrical detection of nucleic acid amplification, and an array of transistors supplied by Taiwan Semiconductor Manufacturing.

*Microfluidic Sample Partitioning*
This thesis has previously demonstrated generation of droplet arrays using a microcapillary pressure injection system (shown in Figure 3.1 D). Initial studies up to 100 dispersed droplets are possible with this arrangement; however, a requirement for a reduction in sample handling demands automated microfluidics to fill each well. Figure 6.1 highlights a scalable, microfluidic approach for automated sample partitioning. This particular design, first introduced by Cohen et al., utilizes a greek-key well that enables complete sample partitioning via flow of LAMP solution followed by flow of an encapsulation fluid such as mineral oil. Mineral oil and the aqueous-based LAMP mix have different phases, densities and are immiscible. This allows for LAMP solution to remain inside the wells while mineral oil forms an encapsulation layer in the microfluidic channel.

The fluidics will be fabricated using standard SU8 photolithography techniques paired with PDMS molding. In this process, a 100-150μm layer of PDMS will be spun onto a SU8 patterned silicon wafer. Due to the vapor permeability of PDMS, a 2-4μm thick vapor barrier of parylene-C will be deposited onto the spun PDMS surface. As discussed in the supplementary information of Heyries et al., a 100μm layer of PDMS/2μm thick parylene-C layer, coupled with a pL reaction volume, will allow 15-20% evaporation. This level of evaporation has not been observed to inhibit LAMP or PCR. To further minimize evaporation, sacrificial water channels can be incorporated in the design to saturate the 100-150μm thick PDMS layer. As a final step, additional PDMS will be poured onto the wafer to ease handling of the microfluidics and simplify tubing integration. Bonding of the fluidics to the silicon FET platform will utilize oxygen plasma bonding.
By using the controlled flow of a syringe pump, optimal flow rates for maximum sample partitioning can be determined. Partitioned samples will then be heated using the heated stage with INSTEC stage controller. Sample evaporation will be monitored by evaluating the change in volume within each well versus time. Modifications to either the spun PDMS thickness, inclusion of sacrificial

Figure 6.1 Sample Partitioning for Digital LAMP: (A) and (B) Schematic overview of sample partitioning combined with an array of field effect transistors within each partition. (C) A design for 9600 individual wells with 6 separate inlets to enable multiplexed detection. (D) Brightfield image of an array of partitioned samples. (E) Close-up view of empty (top) and partitioned (bottom) wells. Each well contains ~300pL of solution.
water channels, or changes to the vapor barrier will be made to ensure the LAMP solution will not be inhibited due to solution evaporation.

Several advantages will come from the division of sample into miniaturized chambers. First, time before diagnosis can be greatly reduced when LAMP amplification is done in a sub-nanoliter volume due to the relative concentration of template. Second, this system has the potential to run multiple assays at the same time for multiplexed analysis. Each channel of chambers will utilize a unique input allowing for multiple sample. Additionally, separate heaters can be devoted for each channel making it possible to run multiple amplifications with different reagents and thermal profiles. Finally, running thousands of wells allows for Poissonian statistics to be applied to the end result of positive and negative wells. Through statistical analysis, advanced by the digital PCR community, absolute quantification of starting template concentration without an associated standard curve is possible. This increases the assays robustness and repeatability, which is essential for a commercial technique.

Localized single cell lysis through nanowire electroporation
As a necessary first step for PCR-based amplification, cells that have been partitioned into individual reaction chambers must have their DNA released from the cell body into the local environment. Traditional methods such as chemical or thermal lysis are not generally suitable for portable diagnostics. Chemical lysis reagents may inhibit amplification resulting in false negatives. Thermal lysis can inactivate the Bst polymerase used in LAMP, rendering the reaction inefficient. These methods can be incorporated with on-chip PCR/LAMP; however, this usually requires a separate purification module that either removes the inhibitory lysis reagents, or keeps the polymerase separate from the heated lysis zone. Nanowire electroporation, on the other hand, allows for direct incorporation of amplification reagents with whole cells. The electroporation method can lyse cells while maintaining compatibility with other reaction components.
Additionally, LAMP is known to be robust in the presence of inhibitors, making it ideal for a direct cell-to-amplification method.(228)

*Electrical detection of PCR by-products*

In order to overcome the need for a traditional optical detection of LAMP or PCR amplification, sample partitioning with single cell electroporation can be combined with the biosensing capabilities of the nanowire transistor. pH changes associated with generation of hydrogen ions by the amplification process offers an attractive means to electrically monitor a reaction’s progress. However, inefficient amplification and yield, as well as buffering used to keep enzymes in the reaction solution at their optimal pH, limit the pH change associated with nucleotide incorporation. To overcome limitations due to amplification yield, we used loop-mediated isothermal amplification (LAMP), an isothermal amplification technique that is known to yield >50x more amplified product than traditional PCR.(225)

This method shows great potential when combined with a solid-state electrode as discussed in Chapter 5. However, it is likely that certain limitations come into play when trying to apply this method to interrogating massively parallel reactions. For starters, the method of pipetting the PVC/DNP in THF mixture onto the chip limits reaction volume resolution. Additionally, if a single electrode is used to interrogate multiple droplets, electrical cross-talk between the reaction volumes could occur through the exposed electrode surface. To combat both of these issues and form a more robust on-chip electrode, future work is focusing on photolithographically patterning the electrode with a pH-insensitive PVC layer. Ideally, this would render the electrode pH-insensitive and isolate it from changes within each reaction volume. In doing so, a single electrode could be used to interrogate multiple reaction volumes through the response of the ISFET within each well instead of a REFET/electrode monitoring system.
System integration with TSMC FET array

Taiwan Semiconductor Manufacturing Corporation has set their sights on becoming one of the world’s largest manufacturers of biosensing FETs. Along these lines, our collaboration with them over the years has been fruitful in developing exciting new microchips for biological testing. Future steps with TSMC will focus on development of a large-scale array for massively parallel testing of FET for biosensing applications. By incorporating microfluidic sample loading, cellular electroporation, and electrical detection of nucleic acid amplification, the FET array will hopefully become the detection platform for the next generation of digital nucleic acid amplification technologies.

In the near term, work will focus on proof-of-concept development of the platform. The pH-passivation of an electrode to enable operation of multiple separated FETs through a single fluid gate electrode still has to be further understood. Additionally, optimization of the sample loading method will be critical to ensure 100% sample distribution uniformity and usage. Variations in sample partitioning can invalidate the Poissonian statistics used for absolute quantification in digital LAMP, making it an ineffective method for diagnostics.

There is a clear and present need for a portable, inexpensive, rapid method for pathogen detection and identification. From point-of-care in the developing world to patient care at the bedside, this sort of technology would revolutionize diagnostics. Hopefully, with the advancements put forth in this thesis and the ongoing work presented, this technology can become a reality.
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Appendix A

Supplementary Information for Chapter 3

Numerical simulation:

The temperature of the droplet was determined by a systematic and self-consistent numerical solution of coupled electrical and thermal responses of droplet. The details are described below.

Calculation of electric field distribution by electrostatic simulation:

In order to calculate the heating of the droplet, we must first calculate the power-dissipation $P = 1/2\sigma E_{ac}^2$, at every point within the droplet, see Eqs. 9, 10. The electric field $E_{ac}$ is obtained by solving the Poisson equation (Eq. 1) numerically for the device shown in Supplementary Fig. 5 by using a well calibrated commercial device simulator [1]. Due to high frequency of applied ac bias (10MHz), the electrostatic screening due to the ac field may be neglected [2], hence $\sigma$ is set to zero in the RHS of Eq. S3. Finally, the source and drain are grounded; therefore we assume the channel potential to be zero (Eq. 5). The solution of Eqs. 3-5 allows us to calculate $E_{ac}$ throughout the device, including the droplet.

Next we calculate the spatially resolved conductivity ($\sigma$) within the droplet by solving for distribution of ion concentration through Eqs. 6-8. The surface charge, $\sigma_{OH}$ is calculated by assuming droplet equals pH 7 and surface OH group density, $N_s \approx 10^{14} \text{ cm}^{-2}$ [3]. Since the potential, $V_{dc}$ due to surface charges (due to formation of double layer) is small (<0.1V), the effective conductivity is essentially identical to that of bulk solution. Regardless, the approach described here is general and should apply to any biasing conditions. Note that the decoupling of the ac and dc
Poisson equation (Eq. 2) is justified because the ac voltage \( V_{\text{rms}}(22-36V) \gg V_{\text{dc}}(<0.1V) \).

**Calculation of temperature distribution by thermal simulation:**

The spatially resolved power dissipation (\( P \)) obtained from the numerical simulation of Poisson equation, is used to calculate the heat generation in the buffer solution (Eq. S10, S11) and the oxide (Eq. S10, S13). Time transient heat equation (Eq. S9) was solved using MATLAB® PDE toolbox [4] to determine the temporal and spatial heat profiles throughout the device. The heat generation terms include both Joule heating of ions in solution as well as dielectric relaxation in water and oxide, see Eqs. 11,13. Also, we assume that the electrical conductivity of the solution is proportional to the ionic concentration (Eq. S12). Convective transfer of heat from the droplet to the air was approximated by assuming that the droplet is covered by a 5μm thick boundary layer of air. Radiative heat transfer, however, was neglected in the simulation. The buffer solution and oxide are assumed to be free of any trap charges. The physical constants used in the simulation are listed in Supplementary Table 2, 4. Dirichlet boundary condition (Eq. S15) was applied on all the outer boundaries for the simulation and thermal fluxes were assumed to be continuous across the interfaces.

**Discussion of the numerical results:**

Supplementary Fig. A.1 shows the electric field profile in the droplet obtained from the solution of Eq. (3) and Eq. (4). As expected, maximum electric field occurs near edges of the active device due to fringing effects. Consequently, the Joule heating of ions is maximum near the surface of the device. Also, as we increase the voltage (a-c), the fringing fields increase and hence, the temperature increases (Supplementary Fig. 1 a-c). The bottom panel in Fig. A.1 a-c shows the
temperature cut at the center of the device along the direction perpendicular to oxide surface.

Simulations for different droplet sizes (Supplementary Fig. A.7), shows that the temperature becomes more uniform as the radius of the droplet decreases. Due to significant mismatch in thermal conductivity of the droplet vs. the substrate, we find that the maximum temperature is essentially independent of the droplet size, i.e.~4 degree change in temperature for 64 times increase in the volume of the droplet. This relative insensitivity of temperature to the droplet size allows precisely tuning of the droplet temperature regardless the inevitable variation in the droplet size.

References:


**Supplementary Figure and Tables**

**Supplementary Figure A.1 Simulations of heating:** (A) and (B) Plots of electric field and temperature for 22 V\text{rms}. (C) and (D) Plots of electric field and temperature for 30 V\text{rms}. (E) and (F) Plots of electric field and temperature for 36 V\text{rms}. (G) Thermal profile within the droplet at X = 0 µm for 20, 30 and 36 V\text{rms}. 

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Supplementary Figure A.2 AC vs. bulk heating evaporation: The AC heating technique shows a 2.5% average decrease in volume ($n = 9$, st. dev. 2.2%). The bulk heating technique shows a 16.7% average decrease in volume ($n = 9$, st. dev. 1.1%) The two-tailed P-value is $< 0.0001$.

Supplementary Figure A.3 Fluorescent images of droplet fluorescence through a voltage sweep: (A) (B) and (C) show fluorescent images of the 50, 61 and 80°C FRET constructs, respectively, as the voltage is increased from 0-42$V_{rms}$. These images represent the data in Fig 4B
Supplementary Figure A.4 SYBR Green melting vs. FRET construct melting: A plot of the melting voltage vs. melting temperature plot with the FRET construct method and the SYBR green method. The melting voltages extracted from SYBR green melting fit the pattern established by the FRET construct melting points.

Supplementary Figure A.5 Cross-sectional view of simulation schematic:

R1: Air

Maximum Heat Generation near surface due to fringing fields

R2: Water

R3: Top Silicon Oxide

R4: Silicon Channel

R5: Bottom Silicon Oxide

R6: Silicon substrate
Supplementary Figure A.6 Maximum temperature uniformity with varying droplet diameter: (A) 3 different diameter droplets were spotted on 3 linked devices. (B) Fluorescence increases uniformly with a voltage sweep. (C) The derivative of (B) is shown. The uniform peak for all 3 droplets implies a uniform maximum temperature in the droplets.
Supplementary Figure A.7 Temperature profile uniformity in droplets of varying radius: Temperature becomes more uniform as the droplet radius becomes smaller.
Supplementary Figure A.8 Uniform, parallel droplet heating: (A) Shows a bright field image of 5 droplets on linked devices where a single lead connects multiple heating elements. (B) A fluorescent image taken before the heating process (C) A fluorescent image taken after heating of the 5 droplets simultaneously. The FRET construct has denatured resulting in an increase in observed fluorescence. (D) Raw fluorescence data taken during the voltage sweep. (E) A derivative of (D) provides the melting voltage for each droplet. The melting voltage is the same for all 5 devices which indicates uniform heating across the linked devices.
Supplementary Figure A.9 Commercial data for the heteroduplex experiment: (A) and (B) show commercial melting curve and derivative data for the FRET constructs used in Fig. 4D and 4E. The heteroduplex shows a melting temperature 6-7°C less than either of the fully complementary strands.
Supplementary Figure A.10 FRET construct dehydration and rehydration:
(A) A schematic of the dehydration/rehydration process. A probe ssDNA sequence is dried on the device surface. The probe ssDNA is rehydrated using low evaporation solution containing the target ssDNA sequence. (B) and (C) The melting curve and derivative of the initial heating-cooling step. The fluorescence increases in dual-peak manner, implying improper initial DNA hybridization.
Supplementary Figure A.11 Dehydration/rehydration of dsDNA FRET construct: (A) Pictures of the process flow are presented. (i) Shows bare device. (ii) dsDNA FRET construct is dehydrated on the surface. (iii) A fluorescence image of the spotted dsDNA. (iv) 3 of the dsDNA spots are rehydrated with the low evaporation Protein Carrier Solution. (v) A fluorescence image of the rehydrated dsDNA. (B) & (C) A melting curve of the rehydrated dsDNA is taken. The fluorescence shows a single distinct peak implying proper DNA hybridization and melting.
Supplementary Table A.1 Description of symbols

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<th>Symbol</th>
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<tr>
<td>$\phi$</td>
<td>Net potential</td>
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<tr>
<td>$\phi_{ac}$</td>
<td>Potential due to applied ac bias</td>
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<tr>
<td>$\phi_{sr}$</td>
<td>Potential due to surface charges</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density of charges</td>
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<td>$\phi_{ch}$</td>
<td>Channel potential</td>
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<td>$\phi_{sat}$</td>
<td>Potential at the bulk contact</td>
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<td>$V_{ac}$</td>
<td>Applied ac bias</td>
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<td>$\sigma_{Si}$</td>
<td>Surface charge due to ionization of Silanol (SiOH) groups</td>
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<td>$\rho_{ion}$</td>
<td>Ionic charge in droplet</td>
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<td>$T$</td>
<td>Temperature</td>
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<tr>
<td>$t$</td>
<td>Time</td>
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<td>$\sigma$</td>
<td>Conductivity in the specified region</td>
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<td>$\sigma_{NaCl}$</td>
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<td>$E_{ac}$</td>
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<td>$T_{air}$</td>
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Supplementary Table A.2 Parameters for electrostatics simulation (Refer to Eqs. (1-8) in Supplementary Table A.3)

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<th>Parameter</th>
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<tr>
<td>Electronic Charge</td>
<td>$q$</td>
<td>$1.6\times10^{-19} \text{ C}$</td>
<td>[5]</td>
</tr>
<tr>
<td>Boltzmann Constant</td>
<td>$k_B$</td>
<td>$1.38\times10^{-23} \text{ J/K}$</td>
<td>[5]</td>
</tr>
</tbody>
</table>
Supplementary Table A.3 Summary of model equations (Refer to Supplementary Table 6-8, Supplementary Figure A.3)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Numerical value/units</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal conductivity of Air</td>
<td>( \kappa_a )</td>
<td>0.024 W/mK</td>
<td>10</td>
</tr>
<tr>
<td>Thermal conductivity of Water</td>
<td>( \kappa_w )</td>
<td>0.58 W/mK</td>
<td>11</td>
</tr>
<tr>
<td>Thermal conductivity of oxide</td>
<td>( \kappa_o )</td>
<td>1.4 W/mK</td>
<td>12</td>
</tr>
<tr>
<td>Thermal conductivity of silicon</td>
<td>( \kappa_s )</td>
<td>149 W/mK</td>
<td>12</td>
</tr>
<tr>
<td>Mass density of air</td>
<td>( \rho_a )</td>
<td>1.2 kg/m³</td>
<td>10</td>
</tr>
<tr>
<td>Mass density of water</td>
<td>( \rho_w )</td>
<td>1000 kg/m³</td>
<td>12</td>
</tr>
<tr>
<td>Mass density of oxide</td>
<td>( \rho_o )</td>
<td>2600 kg/m³</td>
<td>12</td>
</tr>
<tr>
<td>Mass density of silicon</td>
<td>( \rho_s )</td>
<td>2300 kg/m³</td>
<td>12</td>
</tr>
<tr>
<td>Specific Heat Capacity of air</td>
<td>( C_p )</td>
<td>1000 J/kg.K</td>
<td>10</td>
</tr>
<tr>
<td>Specific Heat Capacity of water</td>
<td>( C_p )</td>
<td>4180 J/kg.K</td>
<td>12</td>
</tr>
<tr>
<td>Specific Heat Capacity of oxide</td>
<td>( C_{p,o} )</td>
<td>1000 J/kg.K</td>
<td>12</td>
</tr>
<tr>
<td>Specific Heat Capacity of silicon</td>
<td>( C_p )</td>
<td>710 J/kg.K</td>
<td>12</td>
</tr>
<tr>
<td>Loss factor in oxide at 10 MHz</td>
<td>( \epsilon_{\text{loss}} )</td>
<td>3.9*10^{-2}</td>
<td>9</td>
</tr>
<tr>
<td>Loss factor in water at 10 MHz</td>
<td>( \epsilon_{\text{loss}} )</td>
<td>0.1</td>
<td>9</td>
</tr>
<tr>
<td>Limiting Molar conductivity of Na⁺</td>
<td>( \Lambda_{\text{Na⁺}} )</td>
<td>50 Scm²/mol</td>
<td>14</td>
</tr>
<tr>
<td>Limiting Molar conductivity of Cl⁻</td>
<td>( \Lambda_{\text{Cl⁻}} )</td>
<td>76 Scm²/mol</td>
<td>14</td>
</tr>
<tr>
<td>Surface Silanol (SiOH) group density</td>
<td>( N_s )</td>
<td>5*10^{14} cm⁻²</td>
<td>3</td>
</tr>
<tr>
<td>Ionic concentration of NaCl</td>
<td>( n_\text{NaCl} )</td>
<td>225 mM</td>
<td>-</td>
</tr>
<tr>
<td>pH of buffer solution</td>
<td>( \text{pH} )</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Frequency</td>
<td>( \omega/2\pi )</td>
<td>10 MHz</td>
<td>-</td>
</tr>
<tr>
<td>Calibration parameter</td>
<td>( \alpha )</td>
<td>0.20</td>
<td>-</td>
</tr>
</tbody>
</table>

Supplementary Table A.4 Thermal simulation parameters (Refer to Eqs. (9-15) in Supplementary Table A.3)
Supplementary Table A.5 Averages and standard deviations from Fig. 1
Multiple single droplet denaturation studies were completed using the 50, 61 and 80°C FRET constructs.

<table>
<thead>
<tr>
<th>FRET construct with melting temperature</th>
<th>Average melting voltage</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) 50°C</td>
<td>21.84</td>
<td>1.00 (n=5)</td>
</tr>
<tr>
<td>(2) 61°C</td>
<td>30.3</td>
<td>2.70 (n=8)</td>
</tr>
<tr>
<td>(3) 80°C</td>
<td>36.0</td>
<td>1.81 (n=8)</td>
</tr>
</tbody>
</table>

Supplementary Table A.6 Sequence, melting temperature and melting voltage for the dsDNA fragments used in the SYBR Green denaturation experiment from Supplementary Fig. A.6.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Measured melting temperature ($T_m$)</th>
<th>Measured melting voltage ($V_m$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-AGGCTTAGCTACA-3'</td>
<td>55°C</td>
<td>28.8 $V_m$</td>
</tr>
<tr>
<td>3'-TCCGAATCGATGT-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'-CGCACCAGGCTTAGCTACAAACAT-3'</td>
<td>75°C</td>
<td>33.5 $V_m$</td>
</tr>
<tr>
<td>3'-GCGTGGGCTCCGATGTTTGGTA-5'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'-GGGCGACCAGGCTTAGCTACAAACATGCTGCGATG-3'</td>
<td>82°C</td>
<td>37.2 $V_m$</td>
</tr>
<tr>
<td>3'-GGGCTGGGCTCCGATGTTTGGCAGTGACCCTAAAGTCA-5'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Supplementary Table A.7 Averages and standard deviations from Fig. 4B and 4C. Multiple parallel droplet denaturation studies were completed using the 50, 61 and 80°C FRET constructs.

<table>
<thead>
<tr>
<th>FRET construct with melting temperature</th>
<th>Average melting voltage</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) 50°C</td>
<td>17.04</td>
<td>1.31 (n=5)</td>
</tr>
<tr>
<td>(2) 61°C</td>
<td>26.16</td>
<td>2.15 (n=5)</td>
</tr>
<tr>
<td>(3) 80°C</td>
<td>33.36</td>
<td>1.31 (n=5)</td>
</tr>
</tbody>
</table>
**Supplementary Table A.8 Averages and standard deviations from Fig. 4D and 4E.** Multiple parallel droplet denaturation studies were completed using the 71.7, 70.2, and Heteroduplex of 71.7 and 70.4° FRET constructs.

<table>
<thead>
<tr>
<th>FRET construct with melting temperature</th>
<th>Average melting voltage</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4) 71.7°C</td>
<td>35.76</td>
<td>3.64 (n=5)</td>
</tr>
<tr>
<td>(5) 70.2°C</td>
<td>35.76</td>
<td>2.15 (n=5)</td>
</tr>
<tr>
<td>(4-5) Heteroduplex 64.1°C</td>
<td>28.06</td>
<td>1.07 (n=5)</td>
</tr>
</tbody>
</table>
Appendix B

Supplementary Information for Chapter 4

Supplementary Figures and Table

**Supplementary Figure B.1 Ultra-Localized Lysis:** (a) and (b) are merged brightfield and PI fluorescence images. (a) Shows a grouping of 3 cells above the central nanoribbon device. Only one of these cells (red arrow) is directly above the nanoribbon. (b) After lysis, only the cell that was directly above the device shows a PI fluorescence increase. This implies the lysis of cells is very highly localized to within nanometers of the device surface. Scale bars for (a) and (b) are 40 μm
Supplementary Figure B.2 FRET Construct Melting: A plot of fluorescence increase observed from a 72 °C dsDNA FRET construct denaturing with applied voltage. At the voltages used for irreversible electroporation, 600-900mVpp, no fluorescence increase is observed. This implies that heating is not the likely cause of cell lysis.
Supplementary Figure B.3 Current-Voltage Transistor Characterization Before and After Signal Application: The leakage current from the drain to the bulk (Idb) remains below $10^{-9}$ A before and after cell lysis. The drain-source current (Ids) retains typically transistor characteristics before and after signal application.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Value</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electric Field</td>
<td>$E$</td>
<td>Simulations</td>
<td>-</td>
</tr>
<tr>
<td>Cell Radius</td>
<td>$R$</td>
<td>6.6 μm</td>
<td>1</td>
</tr>
<tr>
<td>Angular Frequency</td>
<td>$\omega$</td>
<td>$2\pi \times 10$ MHz</td>
<td>-</td>
</tr>
<tr>
<td>Capacitance Membrane/Area</td>
<td>$C_m$</td>
<td>$\epsilon_m / \hbar$</td>
<td>-</td>
</tr>
<tr>
<td>Absolute Permittivity of the Membrane</td>
<td>$\epsilon_m$</td>
<td>$7.97 \times 10^{-11}$ F/m</td>
<td>2</td>
</tr>
<tr>
<td>Thickness of the Membrane</td>
<td>$\h$</td>
<td>5 nm</td>
<td>1</td>
</tr>
<tr>
<td>Resistivity of the Interior of the Cell</td>
<td>$\rho_i$</td>
<td>4.5 Ω*cm</td>
<td>1</td>
</tr>
<tr>
<td>Resistivity of the Electrolyte</td>
<td>$\rho_e$</td>
<td>0.769 Ω*cm</td>
<td>3</td>
</tr>
<tr>
<td>Polar Angle with Respect to the Direction of the Field</td>
<td>$\theta$</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Table B.1 Constants for Transmembrane Voltage Calculations
Appendix C
Supplementary Information for Chapter 5

Calculating the Change in pH associated with Nucleic Acid Amplification

This method applies the Henderson-Hasselbalch equation to determine the change in pH associated with a change in hydrogen ions in solution (see equation 1). To start, the buffering components on the solution must be identified, as well as the starting pH. For the Isothermal Amplification Buffer, around pH 7-8, the buffering capacity comes from the 20mM Tris-HCl in solution (pKa 8.08). The starting pH is taken to be pH 8 for these calculations. At this pH, the reaction is below the pKa of Tris-HCl. As the buffering capacity is strongest around the pKa value, it is best to start the reaction below the pKa to ensure the largest potential pH change.

\[
pH_o = pK_a + \log_{10}\left(\frac{[A^-]}{[HA]}\right)
\]

Equation 1: Henderson-Hasselbalch Equation

First determine the starting A\(^-\) and HA concentration for your given Tris-HCl concentration and pH.

At pH\(_o\) 8, pK\(_a\) 8.08, and 20mM Tris-HCl:

\[A^- + HA = 20mM\]

\[A^- = 9.082mM\]

\[HA = 10.918mM\]
For a 500ug/mL LAMP yield, the generated hydrogen ions $[H^+] = 1.515$ mM. For a 40ug/mL PCR yield, the generated hydrogen ions $= 0.121$ mM.

To determine the change in pH associated with generation of hydrogen ions, we apply the Henderson-Hasselbalch equation a second time. In this calculation, the concentration of $A^-$ has been reduced by $[H^+]$. The HA concentration has been increased by $[H^+]$.

$$
\Delta pH = pHe - (pK_a + \log_{10}\left(\frac{[A^-] - [H^+]}{[HA] + [H^+]}\right))
$$

**Equation 1: Delta pH with Henderson-Hasselbalch Equation**

The delta pH for LAMP = -0.136.

The delta pH for PCR = -0.01.

For reaction optimization, the Henderson-Hasselbalch equation states that changing the buffer ($pK_a$), the buffer concentration ($A^- + HA$), the starting pH ($pHe$), or the yield of the reaction ($H^+$) will affect the overall $\Delta$pH.
Supplementary Figures

Supplementary Figure C.1 pH Calibration Curves: The curves shown here depict the changes in pH associated with four NaOH and four HCl additions. Given the pKa of Tris-HCl is 8.08, the curves can be seen to flatten out as they approach pH 8.

Supplementary Figure C.2 O2 Plasma Treatment of On-Chip Platinum: (a) Leak-free reference electrode device response curves without an O2 plasma treatment. The ISFET responds well without O2 plasma treatment when swept with the leak-free electrode. (b) When the platinum is not exposed to oxygen plasma before measurements, the pH sensitivity of the platinum is slow and unstable. (c) and (d) The instabilities of the platinum pH response can also be seen in the open circuit potential measurement of the non-O2 plasma-treated platinum vs. the leak-free reference electrode.
Supplementary Figure C.3 Droplet-in-Oil pH Response: (a) and (b) Depict HCl and/or NaOH additions into droplets-in-oil. To aid NaOH or HCl injection, the droplets were large at 20μL; however, this test proves that droplet-in-oil pH testing is possible with the PVC-REFET plus platinum electrode configuration.
Supplementary Figure C.4 PVC Stability in Mineral Oil: (a) PVC on HMDS without any thermal curing step results in PVC-liftoff at room temperature in mineral oil. (b) PVC on HMDS without thermal treatment, but with an overnight drying step also resulted in liftoff at room temperature in mineral oil (c) Using sigmacote, a common silane for on-chip PCR passivation, resulted in almost immediate liftoff of the PVC. (d) Baking the PVC at 80C provided a significant increase in PVC stability even when heated to 65C for 1 hour.
Supplementary Figure C.5 Endpoint pH-LAMP with a Leak-Free Reference Electrode: (a) and (b) Examples are shown where detection was carried out with a leak-free electrode in solution. As expected, and with the opposite trend to the platinum electrode example, the threshold voltage shifts to a more negative value after amplification. The pH change for these solutions was measured to be ~0.6 units.
Supplementary Figure C.6 Threshold Voltage Stability with Solution Exchange: Two devices were measured, a PVC device (LSP61) and a non-PVC device (LSP94) and the solution exchanged 5 times. Between each solution exchange, the device was rinsed via pipetting 3 times. Minimizing variations in threshold voltage is necessary for robust endpoint detection experiments. Issues such as electrostatic discharge within the device can result in large threshold voltages shifts if they are not controlled. The PVC device showed an average change from step 1 of 1.7mV, st. dev. = 1.26mV. The non-PVC device shows an average change of 0.82mV, st. dev. = 0.16mV. The change is considerably less than the 66mV change seen in the Full amplification result from Figure 5(c).