RICE UNIVERSITY

Integrated Instrumentation and Multivariate Index Assay System for Cardiovascular Health Assessment at the Point-of-Care

by

Michael P. McRae

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE

Doctor of Philosophy

APPROVED, THESIS COMMITTEE

John T. McDevitt, Chair
Brown-Weiss Professor
Bioengineering and Chemistry

Tomasz Tkaczyk
Associate Professor
Bioengineering and Electrical & Computer Engineering

Rudy Guerra
Professor
Statistics

HOUSTON, TEXAS
April 2016
ABSTRACT

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Cardiovascular disease (CVD) is the leading cause of death in the United States and globally, accounting for approximately one in three deaths. Early detection and frequent monitoring of traditional risk factors and biomarkers is necessary to save lives and reduce unnecessary costs due to CVD morbidity and mortality. This dissertation aims to develop new tools and processes that can contribute more effective means to assist in prevention and early intervention at the point-of-care through integrated instrumentation and predictive models for CVD. The Programmable Bio-Nano-Chip (p-BNC) is a platform to digitize biology in which small quantities of patient sample generate immunofluorescent signal that is optically extracted and converted to antigen concentrations. A novel fluid delivery method was developed to provide accurate and repeatable flow rates via actuation of the p-BNC cartridge’s blister packs. A portable analyzer instrument was designed and fabricated so as to facilitate fluid delivery, optical detection, automated data analysis, and intuitive mobile health interfaces, representing a universal system for acquiring, processing, and managing clinical data streams and overcoming many of the challenges facing the widespread clinical adoption of lab-on-a-chip technologies. The p-BNC system is also a biosensor system that learns—a diagnostic device that implements machine-learning algorithms for diagnosis and
prognosis for a variety of disease applications. In this capacity, a Cardiac ScoreCard system was developed to predict a spectrum of CVD using multiplexed biomarker measurements, symptoms, medical history, and demographics. The combination of a platform to digitize biology and predictive analytics has the potential to alter the trajectory of medicine, where the current linear thinking—mainly based on late-stage disease diagnosis using expensive and cumbersome tools—is replaced by a pathway to exponential medicine made possible through the introduction of scalable tools with the capacity to learn.
Acknowledgments

First, I would like to thank my advisor, Dr. John T. McDevitt, for his guidance and support in this work. Through his unbridled enthusiasm, exceptional leadership, extensive collaborative network, and visionary nature, John has created an ideal training environment for scientists and engineers. My unique graduate school experience has been tremendously enjoyable thanks to a skillful balance between quality guidance and academic independence over the past few years.

I would also like to thank committee members, Dr. Tomasz Tkaczyk and Dr. Rudy Guerra, for their expert evaluation and guidance in this work. Special thanks to Dr. Biykem Bozkurt and Dr. Christie Ballantyne for their clinical insights and invaluable feedback on the Cardiac ScoreCard. Thanks to industry partners XACTIV, Open Photonics, MiniFAB, Edmund Optics, and Mobisoft Infotech for helping to make this project a reality and paving the way for these new tools to move closer to real-world clinical practice.

Thanks to McDevitt lab co-workers, Dr. Basil Shadfan, Dr. Eliona Kulla, Ahmed Haque, Dr. Tim Abram, Felipe Romero, Dr. Archana Raamanathan Simmons, and Dr. Nicolaos Christodoulides, who made this a truly enjoyable journey. To Glen Simmons, his efforts in developing the p-BNC cartridge and expert advice for improving instrumentation are instrumental to this work. To Dr. Jorge Wong, his excellent feedback on the software and high-quality illustrations used in this dissertation and other publications are much appreciated. To graduate and undergraduate colleagues Mathews
John, Sanjiv Gopalkrishnan, Matthew Miller, Jack Wang, and Taylor Payne, I am deeply grateful for your significant contributions to this work.

I am very fortunate for my lifelong friends, Carl, Mary, Dave, Katie, Thomas, and all those who I’ve shared many adventures with over the years. I would also like to thank the Valhalla String Band family: Alex, Andrew, Nic, and Maria. Playing music with them has added a new dimension to my life and countless memories during graduate school that I will cherish for years to come. I would like to thank Grace for her constant encouragement and coaching throughout my time in graduate school. Finally, I would like to thank my family, Mom, Dad, and Maggie, for their love and support throughout this journey.
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<td>ACS</td>
<td>Acute coronary syndrome</td>
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<tr>
<td>AI</td>
<td>Artificial intelligence</td>
</tr>
<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
</tr>
<tr>
<td>ANN</td>
<td>Artificial neural network</td>
</tr>
<tr>
<td>ARIC</td>
<td>Atherosclerosis Risk in Communities</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BNP</td>
<td>Brain natriuretic peptide</td>
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<tr>
<td>CA125</td>
<td>Cancer antigen 125</td>
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<tr>
<td>CAD</td>
<td>Coronary artery disease</td>
</tr>
<tr>
<td>CD40L</td>
<td>CD40 ligand</td>
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<tr>
<td>CDSS</td>
<td>Clinical decision support system</td>
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<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
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<tr>
<td>CK-MB</td>
<td>Creatine kinase MB</td>
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<tr>
<td>CMOS</td>
<td>Complementary metal-oxide-semiconductor</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>cTnI</td>
<td>Cardiac troponin I</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DFM</td>
<td>Design for manufacture</td>
</tr>
<tr>
<td>DSA</td>
<td>Double-sided adhesive</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>HE4</td>
<td>Human epididymis protein 4</td>
</tr>
<tr>
<td>HF</td>
<td>Heart failure</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin-1 beta</td>
</tr>
<tr>
<td>IoB</td>
<td>Internet of Biomarkers</td>
</tr>
<tr>
<td>IoT</td>
<td>Internet of Things</td>
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<tr>
<td>IVDMIA</td>
<td><em>In vitro</em> diagnostic multivariate index assay</td>
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<tr>
<td>LED</td>
<td>Light emitting diodes</td>
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<tr>
<td>LOC</td>
<td>Lab-on-a-chip</td>
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<tr>
<td>LOD</td>
<td>Limit of detection</td>
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<tr>
<td>MEMS</td>
<td>Microelectromechanical systems</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>MFI</td>
<td>Mean fluorescence intensity</td>
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<tr>
<td>mHealth</td>
<td>Mobile health</td>
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<tr>
<td>MICE</td>
<td>Multivariate imputation by chained equations</td>
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<tr>
<td>MMP-9</td>
<td>Matrix metalloproteinase 9</td>
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<tr>
<td>MPO</td>
<td>Myeloperoxidase</td>
</tr>
<tr>
<td>MYO</td>
<td>Myoglobin</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>Non-ST elevation myocardial infarction</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal pro-brain natriuretic peptide</td>
</tr>
<tr>
<td>p-BNC</td>
<td>Programmable Bio-Nano-Chip</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PCB</td>
<td>Printed circuit board</td>
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<tr>
<td>PET</td>
<td>Polyethylene terephthalate</td>
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<tr>
<td>POC</td>
<td>Point-of-care</td>
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<tr>
<td>PPV</td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate specific antigen</td>
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<tr>
<td>RANTES</td>
<td>Regulated on activation normal T cell expressed and presumably secreted</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>Soluble intracellular adhesion molecule 1</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST elevation myocardial infarction</td>
</tr>
<tr>
<td>SVM</td>
<td>Support vector machine</td>
</tr>
<tr>
<td>TIMI</td>
<td>Thrombolysis in myocardial infarction</td>
</tr>
<tr>
<td>TMC</td>
<td>Texas Medical Center</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor alpha</td>
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<tr>
<td>UA</td>
<td>Unstable angina</td>
</tr>
<tr>
<td>UK</td>
<td>University of Kentucky</td>
</tr>
<tr>
<td>μTAS</td>
<td>Micro total analysis system</td>
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Chapter 1

Introduction

1.1. Background and significance

In 1971, a team at Intel led by Ted Hoff introduced the first microprocessor, the 4004 “micro-programmable computer on a chip”, launching a new industry and beginning a new era of integrated electronics [1]. When asked by one of Intel’s customers to develop several custom chips for a variety of calculators, Hoff reasoned that it would be easier to manufacture a single general-purpose chip that could be re-programmed through software to perform various tasks. Computers that once occupied entire rooms and required expert operators were now replaced by these tiny devices as they found their way into everyday items such as household appliances, automobiles, games, office

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1 Portions of this Chapter have been adapted from McRae MP, Simmons G, and McDevitt JT. Challenges and opportunities for translating medical microdevices: insights from the Programmable Bio-Nano-Chip. *Bioanalysis* 2016, in press.
equipment, *etc.* The success of the microprocessor is attributed to its small, integrated form factor, ability to simultaneously reduce manufacturing cost and improve performance over existing computers, and value added through new capabilities to a host of commercial products. As a result, the microprocessor not only revolutionized commercial products, but it also profoundly changed the fabric of society by the way we communicate, use information, and interact with our environment.

Lab-on-a-chip (LOC) devices have the potential to transform medicine just as the microprocessor transformed the computer industry. Inspired by the principles of scalability and microfabrication of microelectronics [2, 3], the prospect of performing a wide variety of chem- and bioassays on programmable microelectromechanical system (MEMS)-based sensors is enticing to a broad audience including medicine, food safety, military, veterinary, and consumer wellness. Unlike the computer industry, however, the LOC field requires expertise across a wide variety disciplines—chemical, electrical, mechanical, and bioengineering, materials science, optics, clinical laboratory science, and medicine to name a few. As a result of its multidisciplinary and complex nature, LOC technology development requires substantial initial investments in specialized equipment and human resources. This high barrier to entry has been attributed to LOC technology’s slow uptake since the first significant papers were published in the early 1990s [4, 5].

In the 20th century, breakthroughs in medical and bioanalytical sciences were facilitated by large, government-funded, “top-down” infrastructures. Much like the early days of computing when a single computer occupied an entire room and required expert users to operate, the majority of bioanalytical data today are acquired in similar remote
and central laboratories by trained clinical specialists. While these clinical analyzers are well-suited for high volume, high-throughput, and expertly staffed laboratories, this 20th century solution is ill-equipped to solve 21st century problems like a growing population’s inaccessibility to affordable healthcare, the ever-increasing demand for quality diagnostic information, and the absence of reliable medical infrastructure in developing countries. The idea that healthcare can be personalized by the introduction of miniature medical devices is tantalizing; as such, one of the most attractive uses for LOC devices is for point-of-care (POC) diagnostics. Smaller reagent volumes, faster analysis times, lower power requirements, and higher level of integration are just a few of the numerous advantages POC platforms have over remote and central laboratories [6]. Further, POC devices are both inexpensive and portable, making them accessible to a broader audience than laboratory facilities [7]. The area of POC diagnostics, made possible through LOC technology, embody a 21st century “bottom-up” approach to healthcare where small, easy-to-use, and inexpensive devices are deployed globally to address unmet clinical needs on an individual or small community basis.

The terms LOC/microfluidic are described in the classical micro total analysis system (μTAS) definition [4] as a device intended to analyze a given sample. Lateral flow or immunochromatographic test strips are relatively simple paper-based microfluidic products which have been around for 30-40 years in the form of home pregnancy or glucose test kits and are often considered to be the most successful application of microfluidics. Recent innovations in paper-based microfluidics have resulted in a variety of low-cost, portable, and relatively simple devices for POC applications [8], for example, the works of the Whitesides group [9-12]. In the early 2000s, DNA microarrays
were widely popular and experienced relatively successful translation, e.g., the Affymetrix GeneChip® [13]. In the area of multiplexed protein immunoassays, suspension arrays, such as the Luminex xMap® [14], have seen successful penetration in the clinical laboratory market. While there has been much speculation about the “killer application” for LOC/microfluidics, the POC diagnostics industry is often cited as the most promising application with high-end cancer and molecular diagnostic instruments paving the way for other cost-sensitive devices [15]. In the POC market, the i-Stat® (Abbott) [16] and the Triage® cardiac panels (Biosite) [17] are commercially available and approved for routine clinical use. However, despite the enormous potential of these devices to contribute to society, only a few of these systems have been Food and Drug Administration (FDA) approved and successfully translated from academia and industry to the clinic [18].

The problems of translation for novel LOC systems have been covered extensively in the literature [19, 20]; for instance, the relatively slow uptake of these devices has been attributed to inadequate understanding of economies of scale in mass manufacturing, lack of standardization and integration, and failing to create the “killer app” [21]. While academics often neglect the challenge of technology transfer and deflect this responsibility to their industrial counterparts [22], these practical issues need to be directly addressed early in the design phase. Importantly, researchers developing new LOC technologies must ensure that their device adds substantial value to the target application to maximize clinical utility. Four key challenges remain for the translation of LOC systems to clinical practice [23]:
1. *Integration*: Integrating LOC systems into POC structures that completely replicate the full functionality provided in laboratory settings.

2. *Failure to compete*: Current LOC systems fail to compete with remote and central laboratories in terms of cost and performance.

3. *Need for new content*: LOC systems need to develop new content that adds substantial value to the target application and is currently unavailable at remote and central labs.


In the following sections, these four challenges related to the broad scale release and scaling of LOC systems are discussed.

**1.2. Integration**

The translation of LOC devices from research laboratories to clinical practice remains largely underwhelming, primarily due to a lack of integration between the chip and its peripheral interfaces. This challenge of integration has been documented extensively, giving rise to the pun repeated *ad nauseum* that most LOC devices are merely “chips in a lab” [19, 20], a common reality in which tiny chips rely on cumbersome external equipment and complex interfaces, rendering the chip unusable outside of the academic lab. The following topics pose significant integration challenges to the LOC community: world-to-chip interface, fluid delivery, device programmability, and automation.
1.2.1. World-to-chip interface

A device’s “world-to-chip” interface (i.e., the methods through which fluids such as the sample, capture and/or detection reagents, buffers, and waste are maneuvered in the device) is one of the most important design considerations of a maturing LOC concept. For a LOC device to be considered fully integrated, provisions must be made to reduce the number of fluid handling steps performed by the user. For example, devices that perform “on-chip” pre-processing of the biospecimen are desired over less integrated chips requiring extensive sample manipulation.

Another major consideration for simplifying the world-to-chip interface is the introduction of buffers and reagents to the device. Most immunoassays require a sterile aqueous solution for performing various assay steps. LOC devices often utilize external syringe pumps for introducing fluids to the chip; however, these devices are not practical for clinical use due to size and weight constraints. An elegant solution to these constraints is to embed all the necessary fluids and reagents in the device itself. The challenge is to protect and encapsulate this sterile fluid source within the LOC device in a way that reduces complicated backend manufacturing steps.

A final world-to-chip consideration is how contaminated devices and potentially biohazardous contents are disposed. There is a sustained interest in the LOC community in inexpensive, single-use disposable devices [7], and while it is impractical for some devices which use relatively large liquid volumes, an attractive solution is to implement reservoirs that contain the waste fluids directly on the LOC device.
1.2.2. Fluid delivery

Fluid motivation is one of the most important features of microfluidic systems. Fluids may be manipulated in numerous ways within these devices, e.g., pressure, electric, magnetic, capillary, centrifugal, and acoustic. In the case of bead-based devices, pressure-driven flow plays a critical role on analyte capture [24]. Although capture efficiency is a primary concern for the fluid motivation system, other considerations such as volume limitations and time constraints could impact the flow conditions. Therefore, it is important to develop flexible instrumentation that can cover a range of accurate and repeatable flow rates.

1.2.3. Programmability

Most commercially available LOC devices are relatively simple in design and function, and their target analytes are usually limited to a subset of biomarkers for a particular disease application. Collectively, these devices form a complicated and cumbersome ecosystem targeting single application verticals [25]. There is a lack of broadly responsive bioassay systems with the ability to agilely adopt new disease applications.

1.2.4. Automation

Another major challenge for integration of LOC devices is creating an automated workflow that is compatible for inexperienced users with limited to no human intervention required between sample introduction and data presentation [26]. Automation is necessary not only to eliminate the need for technical training, but also to
reduce the variability between tests and across test sites, allowing for quality standard measurements with a high degree of reproducibility and repeatability.

1.3. Failure to compete

Another major barrier to clinical translation for LOC devices is the failure to compete with remote and central laboratories in terms of cost and performance. In general, POC devices are both more expensive and yield performance inferior to the laboratory [27]. To reach their full potential, it is necessary for these new LOC devices to simultaneously achieve reduced cost and enhanced performance relative to established laboratory methods.

1.3.1. Cost

The microelectronics industry serves as a role model for the diagnostics industry. In particular, the scalability of microelectronics was made possible through the development of novel microfabrication techniques, allowing for massive replication and parallel fabrication of features on the order of microns and smaller. While these techniques inspired the field of microfluidics/LOC early on, there are now many alternative ways to fabricate these devices, both for small quantity production and for mass manufacture. Despite having the requisite technical capabilities to mass produce them, many LOC devices fail to reach clinical utilization simply because manufacturing costs are too high [28]. Further, the expectation of many academic groups is that once a functional prototype is built, industry partners are responsible for the design for manufacture (DFM) process, i.e., the design phase dealing with various issues relating to
cost-effective manufacturability and scalability such as reducing the number of parts, increasing robustness, minimizing assembly steps, and specifying appropriate tolerances [22, 28-30]. This expectation is far from reality if there is any hope that the device will reach clinical realization, and academic groups proposing new LOC devices must play a more active role in the DFM process. This process involves working directly with commercial partners to simplify the design to the least amount of parts possible, modifying features to be more easily manufactured, preparing product requirements and specifications documents, etc. Further, the DFM mentality should begin in the prototype stage (or as early as possible), such that new device concepts are compatible with highly scalable and repeatable manufacturing processes.

1.3.2. Performance

In addition to cost, another stumbling block for LOC technology is performance. Generally speaking, microfluidics/LOC devices represent novel solutions to an already existing market where cost and performance for a given analysis are well-established [28]. Thus, in order for widespread clinical uptake, these new systems must substantially outperform existing technologies in terms of faster analysis times, increased sensitivity, decreased sample volume, better multiplexing capabilities, lower cost, etc. For example, a new device that provides the same results, but much more rapidly, may be attractive for clinical applications where timely diagnosis is important, such as cardiac biomarker detection in the emergency room. Similarly, new devices offering increased sensitivity and lower limits of detection (LOD) are attractive for assays which the increase in sensitivity improves diagnostic performance. Automated POC devices that are
substantially easier to use than their laboratory counterparts may also find opportunities in consumer markets, home monitoring, and resource-limited settings.

1.4. New content

Over the past few decades, biomarker measurements have become ubiquitous to the practice of medicine and play a critical role in clinical decision making. Biomarkers are like keys that unlock valuable information pertaining to patient health that would otherwise be inaccessible. The availability and rapid access to biomarker data is increasingly important for the diagnosis and prognosis of a variety of diseases; however, major inadequacies have resulted in a disappointing rate of biomarker translation to clinical practice over the years. To illustrate this point, from 1995 to 2005 over 26,000 papers were published for cancer and cardiac disease combined, yet only about 1 protein biomarker per year received FDA approval [31]. While this statistic highlights a major gap in biomarker translation from academic and industrial labs into clinical practice, it also illustrates major opportunities for new technologies that will greatly enhance bench-to-bedside translation.

For a clinical community to adopt new technology requires the device to add substantial value over pre-existing methods. Typically, added value of a device is measured based on performance compared to a predicate device for the same application. While substantial improvements in design may overcome existing methods in performance, these improvements may not be enough to convince ultimate clinical
adoption. Rather, LOC developers should also focus on adding new content to maximize clinical appeal.

1.5. Regulatory approval and clinical adoption

Despite large investments in translational research programs, most bioscience research efforts remain largely decoupled from real-world clinical practice. As a result, regulatory approval of groundbreaking medical technologies is at an all-time low [32]. Device development—whether in academia, national labs, or industry—is usually a complicated and lengthy process that occurs in a linear, sub-optimal manner consuming precious time and resources from venture capital and public funding sources. Before embarking on the path to device approval, a number of assumptions are too often made in the absence of evidence that supports the use of the candidate technology for an intended diagnostic target. Such missing information may include invalidity of the intended target itself, absence of a market for its use, or improbability of its adoption as part of clinical practice. Collectively, these shortcomings are frequent causes for product implementation failure and an overall dismal translation rate of such devices from academic and start-up companies to clinical use.

Medical microdevices for advanced screening, diagnosing, and monitoring that are simple to use, rapid, non-invasive, accurate, sensitive, and accessible to the patients in a variety of settings have a better chance at reaching full clinical realization by fulfilling the following requirements: highly controlled clinical samples relevant to the final
clinical usage model, standard operating protocols, electronic data capture system, user needs and specifications, and detailed information related to the regulatory pathway.

1.6. Opportunities for medical microdevice technologies

We are entering a new era of chem- and bio-sensing empowered by exponential advances occurring in a number of disciplines. While the Internet age led to the interconnectedness between people at an unprecedented rate, the next revolution will involve the connectedness of objects—integrating electronics, computing, communications, and transducers to create a smart environment through the “Internet of Things” (IoT). Extending these transformative changes to health care has potential to improve lives globally on an exponential basis. A major missing link in health care today is the absence of the “Internet of Biomarkers” (IoB); that is, consumer-facing clinical testing capabilities with intuitive interfaces that are accessible to individuals, chemists, pharmaceutical scientists, and care-providers, alike. Numerous physical silicon-transducers (accelerometers, gyroscopes, GPS) are already integrated into various mobile devices; however, there is a lack of mobile health (mHealth) connected biomarker measurement platforms that are programmable and can be easily re-tasked for a variety of applications. While nearly 70 percent of current medical decisions are made using diagnostic tests [33], these tests for the most part are currently performed in traditional health care settings using phlebotomists, remote laboratories, delayed reporting, and an

2 The remainder of this Chapter has been adapted from a manuscript submitted to Accounts of Chemical Research.
inefficient workflow that stifles the arrival of exponential technologies with the capacity to transform clinical testing and medical decision making.

Similarly, machine learning has seen explosive growth in recent years due to the emergence of new data mining techniques, the increasing availability of online data, and the decreasing cost of computation [34]. This widespread adoption of artificial intelligent (AI) systems over the past two decades has resulted in a paradigm shift towards data-intensive and evidence-based decision making, spanning a variety of disciplines across government, industry, and academic institutions, alike. Similarly, machine learning is playing an increasingly important role in the chem- and bio-sensing applications and within the practice of medicine; however, despite enormous technological progress, several challenges are preventing AI systems from reaching their full potential [35]. In the context of clinical laboratory measurements, one of the primary barriers is the lack of integration between data acquisition, handling, and interpretation. There is an opportunity for universal and intelligent biosensor systems to significantly improve health care by acquiring, processing, and managing clinical data streams.

The enormous potential of smart biosensor systems is further underscored by trends in both academic and general public interests. Figure 1.1 depicts academic and public interest in various topics over the last decade. While the IoT revolution has gripped the attention of the general population, this enthusiasm is not yet mirrored in academia where only ~1,000 papers in the last few years have been published on the topic. Likewise in academia, the interest in biomarkers has exploded. In the last 10 years, >100,000 biomarker papers were published with the number of biomarker papers
increasing every year. The relative public interest in biomarkers, however, appears to be decaying over time.

Figure 1.1. Academic and public interests in various topics over the past decade; (a.) number of scientific publications reported by the Web of Science (http://www.webofscience.com); (b.) relative popularity of topics in the United States (total searches for the topic out of all Google searches) reported by Google Trends (http://www.google.com/trends).
This disparity between academic and public interests highlights the challenges and opportunities facing new LOC/microfluidics technology. Further, researchers need to convey biomarker information more effectively to the general population, for example, by developing health report cards that convert biomarker measurements into intuitive wellness scores. Additionally, there is an opportunity for researchers to elevate their biomarker work from obscurity by tapping into the popular IoT paradigm. Expanding on the capabilities of consumer electronics, big data analytics, and web-aware sensors, cloud-connected diagnostics can be powerful instruments for wellness tracking and behavior modification. The fusion of data from information-rich IoB and IoT infrastructures with predictive analytics may exponentially improve drug discovery, health policy, and allow new options for personalized wellness management.

### 1.7. Programmable Bio-Nano-Chip system

For the past decade, the McDevitt Research Group has been focused on the development of the Programmable Bio-Nano-Chip (p-BNC) system [36-41]. This multiplex and multi-class platform for bio- and chemical analysis has been demonstrated previously in its ability to assess disease/health status in oral cancer, ovarian cancer, prostate cancer, cardiac heart disease, and trauma using over 22 protein biomarkers, 12 small molecules, and 13 cellular markers [24]. By utilizing the principles of microfluidics and an LOC approach, the p-BNC assays provide a way for monitoring multiple biomarkers simultaneously, require drastically reduced volumes of expensive bio-
reagents, and can provide diagnosis in minutes as compared to the week-long wait times of market available remote lab-based tests.

To illustrate how the p-BNC technology works, Figure 1.2 depicts the bead-based assay system across various length scales. The p-BNC cartridge is a fully integrated, self-contained microfluidic device that has aqueous buffers conveniently embedded for use at the POC. In the current configuration this injection-molded cartridge contains a 4×5 matrix of flow-through microcontainers designed to hold agarose bead sensors. Multiplexing is achieved through spatially programming the bead sensors within the microchip. The six-sided flow-through microcontainer design allows convective transport to the interior of the porous 280 µm diameter agarose beads, where efficient diffusion distances and short depletion layers improve sensitivity relative to flat microfluidic channels [42]. The agarose beads’ 3-D fibrous network provides a distinct advantage over 2-D capture (e.g., lateral flow devices, planar microarrays, 96-well plates) because the 3-D lattice structure allows signal to be layered via a high density of reagent capture. Thus, a higher density of immunocomplexes falls within the optical path of the fluorescence microscope, resulting in higher signal and, thus, improved sensitivity. This mini-sensor ensemble capable of quantitating proteins, small molecules, and oligonucleotides outperforms enzyme-linked immunosorbent assays (ELISA) in terms of limits of detection, analysis time, and usability [38]. Originally inspired by highly scalable and repeatable microfabrication processes, the first p-BNC prototypes were comprised of anisotropically etched silicon 100 wafers. Recent efforts to translate these devices into clinical practice have resulted in mass-produced, globally scalable, inexpensive injection molded plastic cartridges.
Figure 1.2. A graphical depiction of p-BNC features at various length scales. (a.) The approximately credit-card sized p-BNC cartridge. (b.) The p-BNC’s 4×5 bead array with (bottom, fluorescence image) and without (top, SEM image) agarose bead sensors. (c.) Magnified view of a single bead sensor (bottom, fluorescence image) and flow-through microcontainer (top, SEM image). (d.) Porous agarose bead network (SEM image). (e.) Illustration of a typical sandwich immunoassay performed on an agarose bead fiber.

The p-BNC system is a flexible platform for digitizing biology, featuring sensor ensembles that measure biomarkers in highly efficient manner. Figure 1.3 shows the process for a routine cardiac wellness testing application which begins by introducing a small quantity (2 to 100 µL) of patient sample (e.g., blood, serum, or saliva) into a disposable cartridge. The cartridge is inserted into the portable analyzer which automatically performs the multi-step assay sequence. Image analysis routines “digitize biology” by converting the signal into biomarker concentrations. The cardiac biomarker concentration data then flows into disease-specific machine-learning algorithms that have been trained on >1000 patient clinical trials to predict a spectrum of cardiovascular
disease (CVD). The result is a single value “Cardiac Score” which is then displayed to the patient using an mHealth app. Given the tools to track wellness over time, patients learn from their intimate connection with intuitive data streams and are able to play more active roles in managing their cardiac health. This potent combination of high-sensitivity POC diagnostics and machine learning has the potential to transform health care moving forward.

Figure 1.3. The intended use cycle of the p-BNC system for routine cardiac wellness testing.
1.8. Summary and dissertation overview

This dissertation features the development of a flexible biosensing platform and the application of a comprehensive biomarker panel for predicting a spectrum of CVD. Specifically, the development of integrated instrumentation for the p-BNC and machine-learning algorithms are described herein, demonstrating a strong potential to assess early risk and late-stage disease progression for heart attack and heart failure (HF) patients at the POC. Integrating these elements into a streamlined cardiac wellness tool was an enormous systems-level challenge, requiring expertise across many bioengineering-related disciplines including mechanical engineering, electrical engineering, statistics, optics, computer vision, and machine learning. The remainder of this dissertation offers solutions to the significant challenges highlighted in Chapter 1.

This dissertation is organized in the following fashion. Chapter 2 describes a novel fluid delivery method was developed to provide accurate and repeatable flow rates from blister pack actuation, and this work lays the foundation for more integrated instrumentation in Chapter 3.

Chapter 3 details the design of a portable analyzer instrument that integrates fluid delivery, optical detection, automated data analysis, and intuitive mobile health interfaces, representing a universal system for acquiring, processing, and managing clinical data streams. This platform for digitizing biology overcomes many of the challenges facing the widespread clinical adoption of LOC technologies.
Chapter 4 describes the Cardiac ScoreCard system to predict a spectrum of CVD. This *biosensor system that learns* combines multiplexed biomarker measurements, symptoms, medical history, and demographics with machine-learning algorithms for improving disease diagnosis and prognosis.

Chapter 5 concludes the dissertation with closing remarks and perspective on the future of scalable tools with the capacity to learn.
Chapter 2

Fluid Delivery System for Microfluidic Devices with Blister Packs

2.1. Abstract

One of the most important considerations when designing a LOC/microfluidic device is the method of fluid delivery. For POC applications, the compression of external blister packs is an attractive solution because all of the necessary reagents can be stored conveniently on the device. Despite the numerous successful examples—both commercial and academic—of microfluidic devices with blister packs, limited information is available in the literature about controlling volumetric flow rates during blister pack actuation. This Chapter details the development of a fluid delivery system for

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microfluidic devices with blister packs. The system and methods for automating fluid delivery described herein lay the foundation for more integrated instrumentation in Chapter 3.

2.2. Introduction

Fluid motivation plays an integral role in nearly all microfluidic systems that use aqueous solutions to facilitate chemical reactions. As a result, one of the first design considerations for a new LOC/microfluidic device is the method for fluid motivation. Fluid flows can be manipulated in microfluidic devices through a variety of driving forces (e.g., pressure, electric, magnetic, capillary, centrifugal, and acoustic) for many purposes (to mix, react, detect, analyze, separate, etc.) [43]. Previous work has shown the effect of various sample flow rates on analyte capture within the p-BNC system [42]. Briefly, the porous bead substrates have a unique multiple kinetic regime in which the exterior of the bead is reaction-limited while the interior of the bead is transport-limited. Thus, the amount of captured material on the bead largely depends on the delicate interplay between these two effects. Defining the flow rate conditions is an important assay optimization step, and various factors are considered including volume limitations, time constraints, and performance. Therefore, it is important to develop a fluid system that can administer accurate and repeatable flow rates.

Traditionally, microfluidics research has relied on cumbersome equipment like syringe pumps and microscopes which are not amenable to POC settings [6, 44]. Figure 2.1a shows a typical early-stage microfluidics research station featuring external syringe
pumps. Without a compact and fully-integrated fluid delivery system, these research stations render tiny LOC devices into “chips in a lab” that are unusable outside of the academic environment. Among the many possible fluid delivery mechanisms available to LOC developers, the external compression of on-chip fluid-filled pouches, or blister packs, is attractive for POC applications because all of the necessary reagents can be stored conveniently on the device (Figure 2.1b). In this configuration, fluid flow may be initiated by finger actuation [45] or controlled by an analyzer providing blister actuation [46, 47]. Aside from blood glucose measurements, perhaps one of the most successful POC diagnostics to date, the i-STAT® device, uses pneumatic actuation to control the movement of blood sample through a microfluidic cartridge via external electromechanical interaction from a handheld analyzer [16]. Similarly, Daktari Diagnostics is using a blister actuation approach for their CD4+ and CD8+ T lymphocyte counting device [48]. The McDevitt group also has prior experience using blisters for on-chip storage and motivation of fluids [36, 49]. Rigorous control of fluid flow is critical to the accuracy and precision of high fidelity and quantitative bioassays. Further, the ability to customize multi-step assays with various flow rates optimized for reaction kinetics is highly desirable to service a broad range of clinical tests. However, little information is available in the literature about the accuracy and reproducibility of customizable and controlled flows resulting from blister actuation in the context of microfluidic-based bioassay systems.
Figure 2.1. Fluid delivery systems for the p-BNC labcard; (a.) Syringe pump system featuring two external syringe pumps per labcard; (b.) Blister pack fluid storage and delivery system on the p-BNC cartridge with blisters highlighted (blue).

The p-BNC cartridge (described in more detail in Chapter 3) was designed for ultimate convenience at the POC; thus, its liquid reagents are located directly on the cartridge in the form of blister packs which hermetically seal them with foil to prevent contamination and evaporation. These foil packs are simple to manufacture in high volumes and are easily attached to the cartridge with double-sided adhesive (DSA). Figure 2.1b shows the p-BNC cartridge with blister packs highlighted. Upon actuation, the blister packs rupture and release their contents into the cartridge in a controlled fashion. The remainder of this Chapter explains the p-BNC’s fluid delivery system and the two key innovations that ensure repeatability and control of fluid flow in the cartridge. Section 2.3 describes the overall blister actuator system design. Section 2.4
discusses blister burst detection using force sensitive feedback. Section 2.5 presents a geometry-adjusted actuation method for delivering accurate and constant flow rates. Lastly, Section 2.6 ends the Chapter with conclusions and future considerations for blister pack fluid delivery systems.

2.3. Design of a blister actuator fluid delivery system

In order to motivate fluid through the p-BNC cartridge, a compact blister actuation device was developed. Figure 2.2 shows a CAD illustration of a blister actuator module. The module features two size 14 captive linear actuators (part no. 35H4N-2.33-907, Haydon Kerk Motion Solutions, Inc., Waterbury, CT, U.S.A.) to perform linear travel at 0.381 µm per step in microstepping mode (eighth steps). The actuator’s small step size is a critical design feature that allows the blister actuator system to precisely administer smooth fluid flow within the cartridge. Custom 3-D printed parts for the cartridge loading mechanism and supports for the actuators and printed circuit board (PCB) were designed in SolidWorks® 2013 3D CAD software (Waltham, MA, U.S.A.) and produced by 3D Systems ProJet™ 3000 (Rock Hill, SC, U.S.A.). An acrylic base was cut and patterned from a 0.5" thick acrylic sheet (part no. 8560K266, MacMaster-Carr, Elmhurst, IL, U.S.A.) using a CO₂ laser cutter (Universal Laser Systems, Scottsdale, AZ, U.S.A.). Four ¼"-20 carriage bolts (part no. 92356A558, MacMaster-Carr) fastened the actuator support to the acrylic base.
The blister actuator tips were cast in optically clear epoxy, Norland Optical Adhesive 81 (Norland Products Inc., Cranbury, NJ, U.S.A.), from a dome-shaped aluminum mold made via vacuum thermoforming polycarbonate (McMaster-Carr, Elmhurst, IL, U.S.A.). Negative thermoforming molds were milled on a Haas Office Mill 2A CNC machine (Harvey Tool Company, LLC., Rowley, MA, U.S.A.), and a single through-hole was drilled in the center of the cavity for integration with a vacuum. Two 0.5” force sensitive resistors (FSRs) (part no. SEN-09375, SparkFun Electronics, Niwot,
CO, U.S.A.) are embedded between the actuator shaft and the actuator tip for implementing force sensitive feedback.

A custom PCB for hardware integration was designed using PCB Artist™ software and manufactured by Advanced Circuits (Aurora, CO, U.S.A.). The PCB contains two EasyDriver stepper motor drivers (part no. ROB-10267) and Arduino Pro Mini microcontroller board (part no. DEV-11113) from SparkFun Electronics and two LM358 op-amps (part no. LM358NFS-ND) from Digi-Key (Thief River Falls, MN, U.S.A.). An FTDI cable (part no. DEV-09718, SparkFun Electronics) connects the PCB to a laptop for serial communication and power. Graphical user interface (GUI) software was developed in MATLAB® 2014a (Natick, MA, U.S.A.) and compiled as a standalone application using MATLAB® Compiler Runtime.

From an instrumentation perspective, this blister actuator device has several advantages over syringe pumps. One notable advantage is overall size. The blister actuator system takes up nearly 70% less benchtop space compared to a dual syringe pump system. In addition to size, the blister actuator consumes less power (4.5W max) than a syringe pump (9.6W max). While a dual syringe pump system for the p-BNC costs $1500, the parts for a blister actuator prototype cost only $430. With its small footprint and programmable software interface, the blister actuator system makes it easier to run multiple p-BNC cartridges simultaneously at a single work station.
2.4. Blister burst detection

One major challenge for integrating blister packs into an LOC device is the method by which fluids are introduced into the microfluidic cartridge. By design, blister packs are completely sealed to preserve the integrity of their sterile aqueous buffers. Thus, releasing the reagents from a blister pack often requires catastrophic failure of the fully encapsulated blister. In the case of the p-BNC, a puncturing mechanism on the cartridge pierces a foil membrane on the blister upon depression with the actuator tip (Figure 2.3). Once the blister bursts, the reagents are routed into the cartridge through a drain. Recognition of this burst event is crucial to administering accurate volumes and flow rates. To detect the burst event, a novel force sensitive feedback method was developed using a force sensor mounted between the actuator and actuator tip. An algorithm detects when the force suddenly decreases, indicating that the blister has burst and is depressurizing. Once the burst is detected, the software initiates the flow protocol and continues the assay sequence.
Figure 2.3. Line drawing depicting the force sensitive feedback mechanism. As the actuator compresses the blister pack, the increase in internal pressure causes the blister’s foil membrane to deform towards the cartridge’s puncturing mechanism. When the blister is burst, the fluid is routed to a drain in the cartridge’s molded layer. A force sensitive resistor detects when the blister has burst and begins the assay sequence.

2.4.1. Materials and methods

A voltage-to-force calibration curve was generated for each of the FSRs, and a blister burst curve was generated from one of the runs showing force versus actuation distance on right and left blisters. To demonstrate the functionality of the burst detection method, failure rate was calculated from failure mode reports \((n = 794\) assay runs) compiled across 12 different actuator platforms which were designated as “pass” or “fail” based on an expert user’s response.
2.4.2. Results and discussion

Figure 2.4 shows a blister burst curve acquired from both the right and left blister actuators. As the actuator compresses the blister, the force sensed by the FSR increases. When the blister finally bursts, some of the air and fluid evacuates the blister, resulting in a sharp decrease in force sensed by the FSR. A failure rate of 2.77% was observed in 794 assay runs. The most common error \((n = 13)\) was attributed to “uncontrolled fluid release”, which describes a situation where the blister bursts uncontrollably and displaces the sample through the bead array. The next most frequent error \((n = 7)\) was “failure to detect burst”, which is attributed to the force sensitive feedback algorithm failing to identify the burst event. The remaining failures \((n = 2)\) were attributed to “false detection of burst”, indicating a false positive detection of the burst event. The relatively low failure rate observed from this prototype system is promising for future implementation in more integrated instrumentation. Further, this compilation of failure data provides key insights for modifying existing quality control standards and highlights new opportunities for further improvement of the control software.
2.5. Geometry-adjusted actuation rate for controlled fluid delivery

The p-BNC assay uses pressure-driven flow via the actuation of blister packs to perform multiple steps at various programmable flow rates. One challenge with this approach is the difficulty in delivering accurate and constant flow rates due to the geometry of the blister. Figure 2.5 illustrates this issue where constant actuation rates yield flow rates that vary as a function of compression depth. As the actuator compresses the dome-shaped blister, the contact surface area increases; thus, the flow rate increases as actuation into the blister progresses. However, with careful manipulation of the
actuation rate, accurate and constant flow rates can be achieved. In this context, a mathematical model of blister and actuator geometry interaction was developed to deliver constant and accurate flow rates. The dome shape of the actuator tip was chosen such that it approximates the geometry of the blister, thus simplifying the algorithm for geometry-adjusted actuation rate (i.e., by using a dome-shaped actuation surface, contact surface area increases approximately linearly with actuation depth). Another advantageous property of the dome-shaped tip is that a curved actuation surface provides uniform and predictable deformation of the blister, whereas a flat actuation surface caused unpredictable buckling and folding of the blister’s foil.

Figure 2.5. Flow rate profile from a constant actuation rate of a blister pack yields slow initial flow rates that increase as the surface area of contact between the blister and actuation surface increase.
Here, blister actuation was modeled as the interaction of two spherical caps (Figure 2.6) corresponding to the blister and the actuator tip. The total volume of the blister is given by the volume of the spherical cap in Equation 2.1.

![Figure 2.6. Spherical cap geometry.](image)

$$V = \frac{\pi h^2}{3} (3r - h)$$

Equation 2.1. Volume of a spherical cap.

Here, $r$ is the radius of the sphere and $h$ is the height of the spherical cap. To determine the ejection volume as a function of actuation depth, the interaction of two spherical caps was modeled. The actuation depth, $z$, refers to the location of the actuator tip between the apex of the blister ($z = 0$) and the bottom of the blister ($z = h$). Here, the blister and actuator are assumed to have identical geometry; thus, the volume ejected from the blister at actuation depth, $z$, is equal to the interaction volume of two spherical caps at depth $z / 2$ as given by Equation 2.2.
\[ V(z) = \frac{2\pi \left( \frac{z}{2} \right)^2}{3} \left( 3r - \frac{z}{2} \right) \]

Equation 2.2. Interaction volume of two spherical caps as a function of actuation depth.

Then, the volume ejected per step of the linear actuator is found by taking the derivative of Equation 2.2.

\[ \frac{dV}{dz} = \frac{1}{4} \pi z (4r - z) \]

Equation 2.3. Differential equation describing the interaction of two spherical caps.

Lastly, the desired volumetric flow rate, \( Q \), is given by Equation 2.4.

\[ Q = \frac{dV}{dz} \frac{dz}{dt} \]

Equation 2.4. Volumetric flow rate expression.

Here, the desired constant flow rate is generated by applying the actuation rate, \( dz/dt \).

To generate constant flow of reagents, the stepper motor driver sends alternating high and low voltage signals to the motor such that the time delay to the linear actuators, \( dt \), scales with the volume ejected as actuation depth into the blister increases. Flow rates were obtained from five runs, and these time delay instructions were adjusted using a scaling factor to achieve constant flow rate at the desired target.
2.5.1. Materials and methods

To verify the blister actuation algorithm, a method for measuring flow rates was developed. Prototype blisters were fabricated from a dome-shaped aluminum mold made via vacuum thermoforming polycarbonate (McMaster-Carr, Elmhurst, IL, U.S.A.). The blister actuator tips were cast in optically clear epoxy, Norland Optical Adhesive 81 (Norland Products Inc., Cranbury, NJ, U.S.A.), from the same dome-shaped mold. Negative thermoforming molds were milled on a Haas Office Mill 2A CNC machine (Harvey Tool Company, LLC., Rowley, MA, U.S.A.), and a single through-hole was drilled in the center of the cavity for integration with a vacuum. Blisters were filled with 1 mL of blue food dye and closed with DSA polyester film (3M Company, St. Paul, MN, U.S.A.) and another polycarbonate layer. A specialized microfluidic test structure with meandering channels was designed via xurography. Top and bottom capping layers were made from 3M™ AF4300 polyethylene terephthalate (PET), and the meandering channels were patterned in a single adhesive layer of DSA. Geometry-adjusted actuation software was implemented on a standalone blister actuator platform. Five runs at each target flow rate (10, 50, and 100 µL/min) were performed. Blisters were completely actuated for the 50 and 100 µL/min tests and partially actuated (200 µL) for the 10 µL/min tests. Video of the fluid flow (blue dye against a white background) was captured with Canon EOS Revel T1i camera (Canon, Inc., Ōta, Tokyo, Japan).

Video analysis software developed in MATLAB calculated the flow rate by tracking the velocity of the leading edge of the blue dye within a channel of known volume. The resulting flow rates were then signal averaged, and the mean and standard
deviations of the signal averaged flow rates were calculated as well as the coefficients of variation (CV) of the five runs.

2.5.2. Results and discussion

To verify the actuation method, flow rates were measured using a video analysis method. Figure 2.7 shows images of the blister actuation process at various stages. The volumetric flow rates was measured by tracking the fluid velocity of blue dye ejected from prototype blisters using a custom designed microfluidic card with meandering channels (Figure 2.8).

![Figure 2.7. Images of the blister actuation process displaying the actuator compressing the blister at various heights.](image)
Figure 2.8. Screenshot showing automated video analysis of a blister actuation validation experiment. A single-layer PET/DSA microfluidic structure with meandering channels was created to track flow rate of blue dye ejected from a prototype blister. Blue stars overlaid on the frame represent the location of the dye’s leading edge through time as tracked by video analysis software. Volumetric flow rate was calculated by tracking the rate of fluid movement within this characterized structure.

To demonstrate the accuracy and precision of flow rate from the fluid delivery system, high, medium, and low flow rates (100, 50, and 10 μL/min) were targeted by the blister actuator software. These three flow rates represent typical conditions in a p-BNC assay, where sample and reagent delivery are usually performed at slow to medium flow rates (10-50 μL/min) and washing steps are performed at fast flow rates (100 μL/min). Figure 2.9 shows the results obtained in the experimental system using custom video analysis software. The average flow rates measured were 98, 50, and 10 μL/min with CV 4, 4, and 8% for the 100, 50, and 10 μL/min tests, respectively. Relatively low CV and
constant flow rate profiles demonstrate adequate control over fluid delivery using this blister actuation approach.

Figure 2.9. Geometry-adjusted actuation results showing mean flow rates and standard deviation (error bars) for five runs at each target flow rate as the fluid (normalized volume) is ejected from the blister.

2.6. Conclusions

This Chapter presents a novel fluid delivery system that addresses several of the major integration challenges facing medical microdevices as introduced in Chapter 1. One major integration challenge is to design a “world-to-chip” interface that seamlessly integrates fluid introduction and handling steps. The p-BNC solves this problem by adopting a blister pack system which not only prevents contamination and evaporation of
the aqueous buffers for long-term storage, but also provides a means to induce fluid flow. Using a dual blister design, the p-BNC serves both the antigen delivery (right blister) and detecting antibody reagents (left blister) without the use of active valves that would otherwise complicate the design. Another challenge is to develop flexible instrumentation that can accurately and repeatedly deliver a range of flow rates. The blister actuator system presented here demonstrates constant, highly accurate, and repeatable flow rates using a force sensitive burst detection and geometry-adjusted actuation method. A final integration challenge for LOC devices is creating an automated workflow that reduces the number of steps carried out by the user. This programmable fluid delivery system performs fluid handling that would otherwise have been supervised by a trained lab technician using syringe pumps. Further, the automated blister actuation device has the potential to reduce the variability between tests and across test sites, allowing for quality standard measurements with a high degree of reproducibility and repeatability.

While the blister pack actuation approach is attractive for its high level of integration and scalability, device makers transitioning from syringe pumps to this more integrated fluid delivery system should consider the implications of smaller available volumes and slower flow rates on assay performance, particularly during wash steps where high volumes and fast flow rates contribute to better signal-to-noise ratios by more thoroughly washing away unbound reagents.
Chapter 3

Portable Analyzer for the Programmable Bio-Nano-Chip System

3.1. Abstract

The development of integrated instrumentation for universal bioassay systems serves as a key goal for the LOC community; creating an automated workflow compatible for non-experts with limited to no human intervention required between sample introduction and data presentation is a high priority. This Chapter details the p-BNC system, a versatile multiplexed and multiclass chem- and bio-sensing platform for bioscience and clinical measurements. The system is comprised of two main components, a disposable cartridge and a portable analyzer. The customizable single-use plastic

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4 Portions of this Chapter have been adapted from McRae MP, Simmons GW, Wong J, Shadfan B, Gopalkrishnan S, Christodoulides N, and McDevitt JT. Programmable bio-nano-chip system: a flexible point-of-care platform for bioscience and clinical measurements. Lab on a Chip 2015; 15: 4020-4031.
cartridges, which now can be manufactured in high volumes using injection molding, are designed for analytical performance, ease of use, reproducibility, and low cost. These labcard devices implement high surface area nano-structured biomarker capture elements that enable high-performance signaling and are index matched to real-world biological specimens. This detection modality, along with the convenience of on-chip fluid storage in blisters and self-contained waste, represents a standard process to digitize biological signatures at the POC. A companion portable analyzer prototype has been developed to integrate fluid motivation, optical detection, and automated data analysis, and it serves as the human interface for complete assay automation.

In this Chapter, a systems-level perspective of the p-BNC universal biosensing platform is provided with an emphasis on flow control, device integration, and automation. To demonstrate the flexibility of the p-BNC, diseased and non-case patients are distinguished across three significant disease applications: prostate cancer, ovarian cancer, and acute myocardial infarction (AMI). Progress towards developing a rapid 7 minute myoglobin assay is presented using the fully automated p-BNC system.

3.2. Introduction

Significant progress has been made towards the development of LOC devices over the years serving a variety of applications [6, 44, 50, 51]. However, few of these devices have obtained widespread commercial and clinical adoption [3, 52]. Traditionally, microfluidics research has relied on cumbersome equipment like syringe pumps and microscopes which are not amenable to POC settings [6, 44]. Integration of
microfluidic and instrumentation components into a unified system remains a central challenge for POC diagnostics [53, 54]. In particular, developing a portable, inexpensive, and sensitive optical detection system is critically important. Recent innovations in manufacturing high quality optoelectronics like complementary metal–oxide–semiconductor (CMOS) sensors and light emitting diodes (LED) have created new opportunities for low-cost and sensitive instrumentation in LOC devices. Further, co-development of the LOC device and associated instrumentation is essential to ensure mutual compatibility of the integrated system [20, 52]. Similarly challenging is creating an automated workflow compatible for non-experts with limited to no human intervention required between sample introduction and data presentation [26].

Several groups have demonstrated significant progress towards integration and automation of assay systems. For example, Sia and colleagues have developed a low-cost mobile device with laboratory-quality results for testing HIV in remote settings [55]. Cho and co-workers created a fully integrated lab-on-a-disc and portable analyzer that performs simultaneous biochemical analyses and immunoassays from whole blood [56]. The Walt group has designed a platform for automated and multiplexed testing of salivary samples for the diagnosis of respiratory diseases [57]. The Wheeler group developed an immunoanalyzer based on a digital microfluidics platform [58]. Bau and colleagues designed a fully automated reader and a self-contained disposable cassette for nucleic acid detection [59]. Singh and co-workers developed a microchip electrophoretic immunoassay and associated instrumentation for the quantitation of protein biomarkers in saliva [60]. Lee’s group developed a self-powered, integrated microfluidic blood analysis system that eliminates the need for external connections to deliver and analyze whole
blood [61]. Ligler et al. developed an easy-to-use portable array biosensor system that can analyze multiple samples for multiple analytes [62]. Yager’s DxBox performs differential diagnosis of infections using a disposable flow-through membrane immunoassay microfluidic card [63]. Lochhead’s MBio system delivers multiplexed immunoassay results using a novel multimode planar waveguide and a USB-powered reader [64]. Heath’s innovative barcode chip platform accomplishes simultaneous multi-class and multiplexed quantitation of proteins and metabolites from single cells through spatial addressing of capture regions [65]. Qin’s volumetric bar-chart chip performs ELISA using nitrogen gas competition between the sample and an internal control to direct the flow of a visually positive or negative ink bar indicator, overcoming the ambiguity and environmental effects that affect some POC tools such as lateral flow assays [66]. Several groups are integrating mobile phones with LOC devices, leveraging their imaging capabilities, connectivity, and ubiquity for performing various biochemical assays in resource-limited settings. Significantly, Ozcan’s team has developed a mobile phone fluorescence microscope with machine-learning software for automated detection of Giardia cysts [67]. Yager’s work integrates low-cost paper-based devices and cell phone imaging for the goal of increasing access and affordability to healthcare [68]. The Whitesides group has developed a handheld device that performs electrochemical testing and transmits data to “the cloud” using a mobile phone [69]. Demirci and co-workers are using cell phone imaging with microchip ELISA to detect the ovarian cancer marker human epididymis protein 4 (HE4) at the POC [70].

While there are numerous successful applications of LOC devices targeting single application verticals, there remains less progress in the creation of broadly responsive
universal bioassay systems for POC use [20, 25]. The expense and time associated with
design, fabrication, testing, and validation serve as barriers for dedicated single-class
bioassay systems [71]. There is a strong need for universal modular testing platforms
designed for non-expert users that can quickly and efficiently provide the clinical
biosensing community with strategic disease-specific panels.

In this Chapter, a systems-level perspective of the p-BNC [24, 27, 36-38, 41, 49,
72-79] technology is provided. Section 3.3 details the materials and methods used to
develop the prototype analyzer system. Section 3.4 describes the p-BNC assay system
with an emphasis on device integration and automation. Section 3.5 highlights the
versatility of the device by the detection of eight different analytes isolated from human
serum samples across three disease applications, prostate cancer, ovarian cancer, and
AMI. Further, Section 3.6 demonstrates the device’s basic functionality for an in
development rapid myoglobin assay. Lastly, conclusions are presented in Section 3.7.

3.3. Materials and methods

The p-BNC cartridge design was inspired by laminate-based and fully integrated
prototypes described previously [37]. The injection molded plastic disposable cartridges
for this study were manufactured by MiniFAB (Scoresby, Victoria, Australia). Sterile
phosphate buffered saline (PBS) encapsulated in foil blister packs and mounted on the
cartridges served as the washing buffer and on-board fluid source. Glass fiber conjugate
pad was purchased from Millipore (Bedford, MA, U.S.A.) and was cut into 2 × 15 mm
rectangles, spotted with detecting antibody reagents, and embedded in the cartridges.
Capture and detection reagents were prepared with SuperBlock (PBS) Blocking Buffer from Thermo Fisher Scientific (Waltham, MA, U.S.A.). The following were purchased from Edmund Optics (Barrington, NJ, U.S.A.): Nikon CFI Plan Fluor 4× objective lens (part no. 88-378), InfiniTube™ FM-100 (0.5×) tube lens (part no. 58-309), InfiniTube™ FM Nikon CFI60 objective adapter (part no. 58-315), InfiniTube™ mounting C-clamp (part no. 57-788), emission filter (part no. 67-017), four excitation filters (part no. 67-028), z-axis precision compact motorized stage (part no. 88-554), PixeLINK® PL-B957-BL camera (part no. 64-183), 1″ steel post (part no. 59-751), and post holder (part no. 58-976). Four high-power blue (490 nm) LEDs (part no. M490D2), four aspheric condenser lenses (part no. ACL2520-DG15-A), and four SM1 lens tubes (part no. SM1L05) were purchased from Thorlabs (Newton, NJ, U.S.A.). Four LED heat sinks (part no. LPD25-4B), mounting tape (part no. LT-03), and two 350 mA BuckPuck™ DC LED drivers (part no. 3021-D-I-350) were purchased from Luxeon Star LEDs (Brantford, Ontario, Canada). Two size 14 captive linear actuators (part no. 35H4N-2.33-907) were purchased from Haydon Kerk Motion Solutions, Inc. (Waterbury, CT, U.S.A.). Three EasyDriver stepper motor drivers (part no. ROB-10267), Arduino Pro Mini microcontroller board (part no. DEV-11113), FTDI cable (part no. DEV-09718), and two 0.5″ force sensitive resistors (part no. SEN-09375) were purchased from SparkFun Electronics (Niwot, CO, U.S.A.). Two LM358 op-amps (part no. LM358NFS-ND) were purchased from Digi-Key (Thief River Falls, MN, U.S.A.). Four ¼″-20 carriage bolts (part no. 92356A558) and 0.5″ thick acrylic sheets (part no. 8560K266) were purchased from MacMaster-Carr (Elmhurst, IL, U.S.A.). The acrylic sheet was cut and patterned with a CO2 laser cutter (Universal Laser Systems, Scottsdale, AZ, U.S.A.). A 7″ touchscreen monitor (Touch 2)
was purchased from Mimo Monitors (Princeton, NJ, U.S.A.). An embedded PC (part no. GB-BXi3-4010) running Windows® 7 (64-bit) was purchased from Gigabyte Technology Co., Ltd. (New Taipei City, Xindian District, Taiwan). A monochrome CMOS camera (Grasshopper3) was purchased from Point Grey (Richmond, British Columbia, Canada).

A custom PCB for hardware integration was designed using PCB Artist™ software and manufactured by Advanced Circuits (Aurora, CO, U.S.A.). Custom 3-D printed parts (cartridge loading mechanism and supports for actuators, camera board, and PCB) were designed in SolidWorks® 2013 3D CAD software (Waltham, MA, U.S.A.) and produced by 3D Systems ProJet™ 3000 (Rock Hill, SC, U.S.A.). The protective enclosure and supporting frame was designed and manufactured by XACTIV (Fairport, NY, U.S.A.). All software for the analyzer was developed in MATLAB® 2014a (Natick, MA, U.S.A.) and compiled as a standalone application using MATLAB® Compiler Runtime.

### 3.4. The p-BNC system

Figure 3.1 shows the p-BNC assay system comprised of a disposable assay cartridge and portable analyzer. The disposable cartridge (Figure 3.1A) is a LOC microfluidic platform for multiclass and multiplexed quantitation of bioanalytes. The cartridge is self-contained, integrated, and has reagents conveniently embedded for usability at the POC. The labcard device contains analyte-specific antibodies bound to 280 μm diameter agarose beads supported in a plastic flow-through microchip described
previously [80]. This design provides convective transport to the interior of the porous beads, efficient diffusion distances, and short depletion layers which improves sensitivity compared to flat microfluidic channels [42]. Forming immunocomplexes throughout a 3-D matrix allows the signal to be layered via a higher density of reagent capture within a 3-D lattice as opposed to 2-D capture in the case of lateral flow devices. This mini-sensor ensemble is capable of multiplexing fluorescence immunoassays for proteins, oligonucleotides, and small molecules, outperforming laboratory-based ELISA in terms of analysis time, limits of detection, and ease of use [38]. The p-BNC is compatible with both competitive and non-competitive assay formats, and the ability to pick and place bead sensors and reagent pads allows ultimate flexibility in a unified form factor.

The p-BNC analyzer (Figure 3.1B) optically images the p-BNC’s bead sensors and automates sample handling that would otherwise have been performed by a trained lab technician. The automation of sample and reagent handling not only eliminates the need for trained technicians and manual manipulations, but also reduces the variability between tests and across test sites allowing for quality standard measurements with a high degree of reproducibility. Further, the analyzer performs automated data analysis on the acquired images, converts the image into usable data (*i.e.*, biomarker concentrations), and relays the data to disease-specific machine-learning algorithms for diagnosis and prognosis. Since multiple disease applications are represented in this work, an in-depth description of these diagnostic and prognostic algorithms and their clinical interpretation is out of scope.
3.4.1. Disposable p-BNC cartridges

The labcard device featured in this work was created in two major stages. First, laminate-based prototypes were developed using a rapid prototype approach [37]. More recently, an injection molded version of the laminate prototype was developed in collaboration with commercial partners where the manufacturing processes established to date are compatible with scaling up to high volume and automated production. Figure 3.2 depicts the cartridge’s major features and internal microfluidic circuitry. The main fluidics module consists of an injection molded cartridge body capped with adhesive layers designed for scalable manufacturing. The bio-specimen (e.g., serum or saliva) is introduced into the sample loading port. Fitted to the main fluidic body, this plastic piece is the inlet and reservoir for sample introduction. An easy-to-use adhesive cap is tethered
to the loading port and sealed prior to initiating the assay sequence. The user-configurable reagent pad chamber is accessed through an opening on the top face of the card, allowing the assay to be easily reconfigured via a conjugate pad with dried reagents (e.g., detecting antibody). The reagent pad is a glass fiber conjugate pad cut into 2 × 15 mm rectangles. The glass fiber material serves as a high surface area support for the detecting antibodies. The pad’s high aspect ratio facilitates repeatable and efficient elution of the reagents. Two foil blister packs containing buffers serve as the on-chip fluid source. Blister puncturing mechanisms on the molded layer rupture the blisters upon actuation and facilitate fluid flow into the cartridge. Self-contained waste chambers on either side of the bead sensor array store the used reagents and bio-specimen for safe and convenient disposal of the device. The sensor region shown here is comprised of a 4 × 5 array of flow-through microwells which are “programmed” by the placement of analyte-specific agarose bead sensors. The chip wells are tapered hexagonal containers; the six walls have a tapered angle of 54.7° with respect to the normal and are designed to contain a single 280 μm bead. This critical design feature allows for pressure-driven flow to deliver analytes into the porous beads via convective transport, effectively layering signal within the 3-D agarose lattice [24]. While larger bead array matrices have been explored in the prototyping phase of development, the 4 × 5 matrix was selected based on a variety of factors. For instance, the well spacing is comfortably within plastic injection molding manufacturing capabilities, and this particular configuration fits within the desired field of view of the analyzer. For cell counting and differentiation applications, this sensor modality is simply swapped with a membrane capturing element (not shown). The cartridge shell protects the blisters during shipping and handling, contains alignment
features for mechanical stabilization within the portable analyzer, and provides a surface for the cartridge label which contains patient ID, cartridge serial number, and a unique QR code for identification.

The dimensions of the cartridge are largely dependent on the volume of sample and reagents necessary for the assay. The sample loop was designed to facilitate 100 μL of sample, and the self-contained waste chambers were designed to store the sample volume and about 2 mL of buffer from the blister packs. Further, the dimensions and positioning of the cartridge components allows for external manipulation of the blister packs via the actuators without interfering with LED excitation and optical instrumentation. Similarly, the bead array is centered between symmetrical waste chambers so that the optical assembly may be centered within the instrument. The sample loading port is located opposite the bead sensors so that it hangs outside of the instrument, minimizing the risk of leakage and potential contamination inside the instrument. A more detailed description of the cartridge design will be covered in a future report.
Figure 3.2. Drawings of the p-BNC disposable cartridge in (a.) an exploded view and (b.) assembled top view.

3.4.2. Portable analyzer

The p-BNC analyzer is approximately 9” × 9” × 12” (length × width × height), weighs less than 15 lbs, and has four main functions: fluid motivation, optical detection,
automated data analysis, and human interface. Figure 3.3 highlights the major components that facilitate these functions.

Figure 3.3. Schematic illustration highlighting the p-BNC analyzer’s major features.

3.4.2.1. Optical detection

The p-BNC analyzer uses a compact fluorescence microscope system for imaging fluorescently labeled beads. The illumination module consists of four equally spaced,
obliquely oriented (45° from normal) blue LEDs (490 nm) that are filtered (482.5 nm center wavelength bandpass filter) and relayed to the p-BNC chip via aspheric condenser lenses with a diffuser surface. One of the main challenges in using an off-axis illumination system is achieving uniform illumination intensity across the entire bead array. Illuminating with four symmetrically spaced LEDs offered superior uniformity over 2- and 3-LED configurations. Additionally, improvements in signal-to-noise and signal-to-background ratios have been observed with increased illumination power, suggesting the potential for improvements in sensitivity at low concentrations. Since diffuser surfaces in the aspheric condenser relay lenses limited the illumination power in favor of increased uniformity, multiple LEDs were necessary to provide adequate illumination intensity. The compact lens assembly consists of an objective lens, emission bandpass filter (534.5 nm center wavelength), and tube lens. An off-the-shelf objective lens was selected with 4× magnification and 0.13 NA for its availability, performance, and form factor. Similarly, the tube lens provides a convenient in-line optical assembly, condenses optical path length, and demagnifies the image (0.5×). A monochrome CCD camera (Sony ICX285 image sensor) was selected for its low-profile board-level configuration and USB compatibility. While the analyzer featured here uses a CCD imager, the modular optical assembly can accept cost-effective and high-performance CMOS image sensors such as the Sony IMX174. The optics module is mounted to a linear motorized stage that translates the entire assembly for focusing. An autofocusing algorithm works by translating to the vertical position with maximum variance in a region of interest (ROI) containing the positive calibrator beads as focal aids.
3.4.2.2. Automated data analysis

The p-BNC’s image analysis software implements novel computer vision methods for detecting the location of beads using a Gabor annulus approach and analyzing pixels according to various regional and intensity-based parameters. The Gabor annulus method, based on the advantageous properties of Gabor wavelet filters, is a popular method for recognizing patterns [81]. A filter for recognizing spherical beads is defined by Equation 3.1 and Equation 3.2 in which a Gabor filter is wrapped around the origin.

\[
G(x, y) = \frac{1}{2\pi\sigma r_0} e^{-\pi \left[ \frac{(r-r_0)^2}{\sigma^2} \right]} e^{i[2\pi f_0 (r-r_0)]}
\]

Equation 3.1. Gabor annulus filter.

\[
r = \sqrt{(x-x_0)^2 + (y-y_0)^2}
\]

Equation 3.2. Expression for radius of Gabor wavelet.

In Equation 3.1, the first exponent is an elliptical Gaussian with standard deviation \(\sigma\). The second exponent is a complex wave with frequency \(f_0\). The radius of the annulus is specified by \(r_0\). In Equation 3.2, \(x_0\) and \(y_0\) specify the coordinates of the filter’s origin. The resulting filter is convolved across the image, resulting in a large response in areas where an image’s features or patterns match their specific scale, orientation, and location.
In other words, the filters are fine-tuned to recognize beads, and the alignment of the beads and the Gabor annulus filter results in a large response at the beads’ centroids.

Figure 3.4 shows the main steps of the image analysis process that is applied to each bead individually. First, the derivative of the raw image isolates the bead’s edge from the background and the bead interior. Then, multiple Gabor annuli are formed with slight variations in radius, frequency, and standard deviation parameters. Using a range of Gabor annulus filters with different properties provides broader coverage for recognizing underlying patterns in the image [82]; here, the wavelet properties are tuned to account for the size distribution of beads. The filters are convolved across the image, and the convolution responses are normalized and aggregated. The centroid of the bead is determined by locating the maximum aggregate response. The bead’s outer radius is then mapped by revolving an intensity profile about the bead centroid and fitting a circle to the resulting coordinates that correspond to the greatest rate of change in pixel intensity. The distribution of signal across a bead is typically nonuniform, exhibiting strong signal along the outer radius and weak signal towards the center. Averaging the pixels over the entire bead region leads to lower signal and higher intra-bead variance. Therefore, an annular ROI method was developed to exclude low signal pixels in the bead center and instead analyze the pixels around the edges of the bead. Once the ROI is mapped, the mean signal is extracted for each bead, and a two-sided Grubbs’ test for outliers is applied to the redundant bead sensors using a significance level of \( \alpha = 0.10 \).
Figure 3.4. Summary depicting major steps of the automated image analysis routine for a single bead. A collection of Gabor annulus filters are applied to the derivative of a raw image via 2-D convolution to locate the centroid of the bead. Once located, the annular ROI method averages the pixel intensity near the bead’s outer radius where signal is greatest (the ROI shown between red and blue circles).

3.4.2.3. Human interface

The cartridge slot contains a 3-D printed alignment feature that accepts the disposable and snaps the cartridge into precise alignment with the blister actuators and optics module. The portable reader also features a touchpad user interface, a fan and vents for cooling, and a removable rear panel with electronic interfaces for the following: barrel power inputs for powering the embedded PC and LED module, two USB A-A
connectors, an RJ45 adapter for ethernet connection, and an HDMI adaptor for displaying on an external monitor. Data may be transmitted to a server via wireless internet or manually via USB. The exterior of the p-BNC instrument supports, encloses, and protects the instrumentation modules from the environment and prevents background light from entering the analyzer.

### 3.4.3. Device operation

With the bead sensors pre-loaded into the cartridge, the user deposits $\geq 100 \mu$L sample (serum, saliva, or urine) into the sample loading port and seals the cartridge cap. The cartridge passively wicks the sample through the main fluidic channel and precisely meters $100 \mu$L to be used in the assay. The user then inserts the card into the analyzer and starts the assay in which the remaining steps are performed automatically by the portable analyzer. The sample delivery is controlled by compressing the right blister while the detecting antibody delivery is controlled by compressing the left blister. To release the blister contents, the blister actuator steps down towards the cartridge until the FSR detects the top of the blister. The actuator compresses the blister until the blister’s foil is ruptured by the sharp puncture mechanism underneath the blister. The bursting event is detected by sensing a change in voltage across the FSR. Next, the blister actuator compresses the blisters according to a geometry-adjusted actuation rate.

The analyzer can perform an extensive menu of assays with customizable flow protocols. The two general fluid profiles are incubation and washing. Incubation steps utilize slow flow rates (1-50 $\mu$L/min) to allow adequate binding between antibodies and antigen in the sample. Wash steps are performed at faster flow rates (100-250 $\mu$L/min) to
remove unbound reagents from the sensor array. Once the assay steps are complete, the chip is imaged using the analyzer’s compact fluorescence microscope. The analyzer’s software performs automated image analysis to convert bead signal intensity to biomarker concentration, and these concentrations are then relayed to machine-learning algorithms for disease diagnosis and prognosis. After completion of the measurement, the used cartridge, which has built-in and self-contained waste storage, is ejected from the analyzer and can be placed in an appropriate biohazard waste container.

This p-BNC technology has many potential applications spanning a diversity of consumers. Among these envisioned markets is the R&D sector which requires flexibility to develop customized biomarker panels. The p-BNC provides this flexibility via an “open-source” format in which the device “programming” steps are completed by the R&D consumer. Currently, this programming step involves manually loading the beads into the cartridge using tweezers and a dissecting microscope and depositing a glass fiber conjugate pad into the reagent pad chamber. Automated bead placement technology for fully developed assays is currently in development, and R&D users are expected to take advantage of both automated and manual methods for programming the cartridges. The p-BNC chip has microcontainers for 20 beads. Theoretically, 20 different biomarkers could be assayed simultaneously; however, the multiplexing ability of the p-BNC is limited by the specificity and cross-reactivity of the assay developer’s reagents. It is also advantageous to have biomarker redundancy in the panel because averaging signal from multiple beads improves the accuracy and precision of the assay. While blister packs containing PBS are currently mounted on the cartridge during the manufacturing process,
future R&D applications may require customizable blister packs sold separately and mounted to blank cartridge bodies with DSA.

3.5. Demonstration of device multi-functionality

One major hurdle for clinical translation and widespread adoption of a new LOC device is its ability to be easily re-tasked for new applications. To demonstrate the bioassay functionality of the p-BNC assay system, a proof of concept study was performed using the integrated and automated bioassay platform. In development assays for prostate cancer screening, ovarian cancer screening, and AMI diagnosis were performed using human serum samples for cases and controls. The purpose of this study is to show proof of concept for an integrated and automated bioassay platform with similar performance to previous work conducted in a less integrated (i.e., “macro-scale”) manner [24]. As such, accuracy and reproducibility studies for a broad coverage of clinical applications on this new instrument is out of scope at this time and will be featured in future publications.

3.5.1. Materials and methods

Using the p-BNC disposable cartridges and prototype analyzer, human clinical samples were tested for prostate cancer, ovarian cancer, and AMI. Informed consent was obtained from all participating subjects prior to testing. In each disease area, “case” and “non-case” patients were analyzed using multimarker panels. For prostate cancer, human serum samples were obtained from a female control and a suspected prostate cancer male patient. The prostate cancer panel, containing total and free prostate specific antigen (PSA) bead sensors, attempts to distinguish between healthy and suspected prostate
cancer cases. Mouse monoclonal anti-human total PSA (catalog no. 10-1122) (Fitzgerald Industries International, Acton, Massachusetts, U.S.A.) and free PSA (catalog no. M167) (CalBioreagents, San Mateo, CA, U.S.A) antibodies were conjugated to the bead sensors by reductive amination for target capture. Matched pairs (catalog no. 10-1123) from Fitzgerald Industries International were conjugated to AlexaFluor-488® for detection using the AlexaFluor-488® protein labeling kit from Life Technologies (Carlsbad, CA, U.S.A.) following manufacturer protocols. For ovarian cancer, human serum samples were obtained from a recent study, and a 2-plex panel was developed using protocols previously described [37]. The panel, containing cancer antigen 125 (CA125) and HE4 bead sensors, attempts to distinguish between a healthy female control and a late-stage ovarian cancer patient. Human serum samples for cardiac testing were obtained from a recent clinical study following chest pain patients who presented to the emergency room with symptoms of acute coronary syndrome (ACS). The cardiac panel, containing bead sensors for cardiac troponin I (cTnI), creatine kinase MB (CK-MB), myoglobin (MYO), and N-terminal pro-brain natriuretic peptide (NT-proBNP), attempts to distinguish a non-case chest pain patient and a patient diagnosed with AMI. Mouse monoclonal anti-human antibodies were conjugated to the bead sensors by reductive amination for target capture, and matched pairs were conjugated to AlexaFluor-488® following manufacturer protocols. Further details of the cardiac assay will be reported elsewhere. For each disease application, serum was separated from whole blood at the respective clinical sites and stored in aliquots at -80°C. The undiluted samples were thawed immediately before introduction to the cartridge, and 100 μL of serum was used for each assay.
All fluid manipulations were handled by the portable analyzer. Case and non-case assays were performed once for each disease application. Images were captured using an exposure time of 500 ms for prostate cancer and 100 ms for cardiac and ovarian cancer panels. Median pixel value of the background, defined as the entire image excluding the bead sensors, was subtracted for all images. Display intensities range 0 to 1000 for ovarian cancer and 0 to 2000 for cardiac and prostate panels. Mean fluorescence intensity (MFI) was calculated for each biomarker type via an annular ROI method where the signal was averaged within the annulus between the bead outer diameter and 90% of the bead radius.

3.5.2. Results and discussion

To demonstrate the p-BNC system’s multi-functionality, a series of in development assays were performed using human serum samples covering three distinct disease applications: prostate cancer, ovarian cancer, and AMI. Figure 3.5 shows data collected for each disease area. The prostate cancer panel (Figure 3.5a) shows stronger signal from total PSA beads in the suspected prostate cancer patient than the female control and modest increases in free PSA signal. This proof of principle experiment demonstrates the assay’s ability to discriminate prostate cancer from healthy controls. In the ovarian cancer panel (Figure 3.5b), HE4 exhibits substantially higher signal for the late-stage ovarian cancer patient versus the healthy control while showing slightly lower signal in CA125. This example highlights the importance of multiplexed panels in the diagnosis of ovarian cancer. While CA125 is considered the gold-standard marker for prognosing ovarian cancer, a single-marker CA125 test may have reported a false
negative result for this late-stage ovarian cancer patient. In the four-plex cardiac panel (Figure 3.5c), increases in fluorescence intensity are observed between the non-case chest pain patient and the AMI patient for all cardiac biomarkers (cTnI, CK-MB, MYO, and NT-proBNP). This suggests that the p-BNC cardiac panel could detect AMI from non-case chest pain patients in the emergency room. One limitation of the current p-BNC cartridge is that it is not yet configured for interrogating whole blood. Modifications to the cartridge design are necessary to facilitate whole blood analysis. Although qualitative and in need of more rigorous characterization efforts to show actual clinical utility, this compilation of data reveals the flexibility inherent in the p-BNC system in which different configurations of bead ensembles can be used to reprogram the chip so as to cover alternative disease indications.
Figure 3.5. Multi-functionality of the p-BNC system showing data obtained with injection molded cartridges and portable analyzer prototype for three clinical applications: prostate cancer screening (a.), ovarian cancer screening (b.), AMI diagnosis (c.). Fluorescent photomicrographs (i. and ii.) represent non-cases/controls and cases, respectively. All three devices include positive (bright beads) and negative controls (dark beads) at the left most and right most extremes of the bead array (not outlined). Two biomarkers were measured for prostate cancer: total PSA (blue) and free PSA (yellow); two for ovarian cancer: CA125 (red) and HE4 (green); four for the cardiac panel: cTnI (red), CK-MB (green), MYO (blue), and NT-proBNP (yellow). Mean fluorescence intensities (iii.) were calculated via the annular ROI method. Error bars show the standard deviation from redundant bead sensors from a single experiment (i.e., the intra-assay standard deviation).
3.6. Rapid myoglobin assay

Myoglobin, a biochemical marker of myocardial injury, is useful for diagnosing AMI due to its rapid kinetics relative to other cardiac biomarkers [83-85]. Serum myoglobin levels may be elevated within 1 to 2 hours of myocardial death while other markers like CK-MB and cTnI appear 3 to 6 hours after symptom onset [86]; however, myoglobin has low specificity for cardiac necrosis, necessitating the inclusion of more specific cardiac biomarkers in the final cardiac panel. While the characterization of this full cardiac panel will be presented in future work, here initial progress is shown for a rapid myoglobin test on the p-BNC system.

3.6.1. Materials and methods

MYO-specific antibodies and standards were acquired from Meridian Life Sciences Inc. (Memphis, TN, U.S.A.). AlexaFluor-488® was conjugated to MYO (clone 7C3) using AlexaFluor-488® protein labeling kit (Invitrogen, Eugene, Oregon, U.S.A.). Goat anti Mouse IgG (H + L) (R-PE) antibodies from Fitzgerald Industries International were conjugated to the beads as positive controls. Reagent pads were functionalized with a cocktail of detecting antibody reagents and installed in the cartridge’s reagent pad chamber. For the dose curve, twelve MYO beads, four positive calibrator beads, and four negative calibrator beads were loaded by hand into the 4 × 5 chip. All fluid manipulations were handled by the automated portable analyzer prototype. Images were captured using exposure time of 1000 ms. Median pixel value of the background was subtracted for all images. MFI was calculated for each biomarker type via an annular ROI method where
the signal was averaged within the annulus between the bead outer diameter and 90% of the bead radius. A dose response curve (5-parameter logistic) was constructed for MYO using a concentration range 0 ng/mL to 2500 ng/mL, and the MFI was recorded for a single run for each dilution, and intra-assay standard deviation was calculated from 12 redundant MYO bead sensors. LOD was estimated from three intra-assay standard deviations above background signal derived from a blank run. For the precision study and correlation study, four of each bead type (MYO, CRP, positive calibrator, negative calibrator, and focal aid) were included. Intra- and inter-assay precision was assessed via replicate (N = 5) measurements of Liquichek™ Cardiac Markers Plus Control (Bio-Rad Laboratories, Inc., Hercules, CA). A correlation between the p-BNC system and the gold-standard reference method (LUMINEX) was established using samples (N = 12) from the cardiac study described in Section 3.5.

3.6.2. Results and discussion

In Figure 3.6 the dose response curve for myoglobin shows an excellent fit to the 5-parameter logistic curve ($R^2 = 0.9998$) with LOD of 1.96 ng/mL. In Figure 3.6, inter-assay precision (CV) for the positive calibrator, negative calibrator, myoglobin, and C-reactive protein (CRP) sensors was 6.99, 12.05, 12.07, and 8.91%, respectively. Clinical samples (N = 12) measured on the p-BNC system and LUMINEX show good correlation ($R^2 = 0.8480$) between platforms for this in development assay (Figure 3.8). Although preliminary in nature, these results suggest that measurements within the relevant physiological range can be obtained with the low-cost, portable p-BNC system in much less time (7 minutes) than gold-standard reference methods (hours to days).
Figure 3.6. Rapid myoglobin assay dose response curve shows the MFI for a single run at various dilutions and intra-assay standard deviation from 12-fold bead sensor redundancy (error bars).
Figure 3.7. The precision bar plot shows the mean fluorescence intensities for each of the five runs (colored bars) across all four bead types and their intra-assay standard deviations (error bars).
Figure 3.8. The initial correlation plot displays LUMINEX versus p-BNC measurements for 12 cardiac patient samples.

3.7. Conclusion

A portable, cost-effective, and sensitive detection system was developed that is capable of remote multiplexed and multiclass clinical testing via standardized diagnostic test ensembles that can be quickly customized for new applications. The p-BNC cartridge has successfully transitioned from a prototype device produced in modest volumes to a device designed for mass manufacturing. Further, a portable analyzer prototype was developed for non-expert users and fully automates otherwise complex assay protocols,
fluid motivation, optical detection, and data analysis. The p-BNC’s versatility was demonstrated by quantifying eight analytes across three different disease applications. Although preliminary and in need of further characterization in clinical settings, the p-BNC system described here has the potential to provide quality multiplexed and multiclass POC measurements for a variety of disease applications.

Recently, the McDevitt group has coordinated with commercial partners to complete the design and manufacture of 10 portable analyzers (Figure 3.9). Inspired by the original prototype in this Chapter, these new instruments feature several design improvements and are compatible with scalable manufacturing practices. With their ability to serve a variety of clinical applications, these ultra-flexible and universal portable analyzers have the potential to penetrate multiple market sectors and improve the quality of health care globally.

While Chapters 2 and 3 describe a flexible platform for digitizing biology, the next Chapter establishes the p-BNC as sensors that learn—in other words, combining the p-BNC’s biomarker measurement data with machine learning algorithms. The potent combination of high-sensitivity POC diagnostics and machine learning has the potential to transform health care moving forward.
Figure 3.9. The recently developed p-BNC platform streamlines multimarker measurements with disease-specific machine-learning algorithms, such as the Cardiac ScoreCard, to provide intuitive indices of health status at the POC. The p-BNC analyzer is shown both with (left) and without (right) the enclosure.
Chapter 4

Machine-Learning Approaches towards the Development of the Cardiac ScoreCard

4.1. Abstract

Clinical decision support systems (CDSSs) have the potential to save lives and reduce unnecessary costs through early detection and frequent monitoring of both traditional risk factors and novel biomarkers for CVD. However, the widespread adoption of CDSSs for the identification of heart diseases has been limited, likely due to the poor interpretability of clinically relevant results and the lack of seamless integration between

measurements and disease predictions. This Chapter presents the Cardiac ScoreCard—a multivariate index assay system with the potential to assist in the diagnosis and prognosis of a spectrum of CVD. The Cardiac ScoreCard system is based on lasso logistic regression techniques which utilize both patient demographics and novel biomarker data for the prediction of HF and cardiac wellness. Lasso logistic regression models were trained on a merged clinical dataset comprising 579 patients with 6 traditional risk factors and 14 biomarker measurements. The prediction performance of the Cardiac ScoreCard was assessed with 5-fold cross-validation and compared with reference methods. The experimental results reveal that the ScoreCard models improved performance in discriminating disease versus non-case (AUC = 0.8403 and 0.9412 for cardiac wellness and HF, respectively), and the models exhibit good calibration. Clinical insights to the prediction of HF and cardiac wellness are provided in the form of logistic regression coefficients which suggest that augmenting the traditional risk factors with a multimarker panel spanning a diverse cardiovascular pathophysiology provides improved performance over reference methods. Additionally, a framework is provided for seamless integration with biomarker measurements from POC medical microdevices, and a lasso-based feature selection process is described for the down-selection of biomarkers in multimarker panels.

4.2. Introduction

The condition of CVD is a diverse class of diseases affecting the cardiovascular system. Although mortality rates are declining somewhat, CVD remains the leading
cause of death and serious illness in the United States, accounting for nearly one of every three deaths [87]. With staggering direct and indirect costs, CVD is a major contributor to rising healthcare expenditure in the U.S. For instance, HF costs alone are projected to double by 2030, with every US taxpayer paying up to $244 each year [88]. The most common type of CVD is coronary artery disease (CAD), which is characterized by atherosclerotic plaque buildup that begins early in life and slowly progresses over time. About 50% of cardiovascular deaths occur due to sudden cardiac death, and a vast majority due to CAD [89]. In a significant proportion of these events, sudden cardiac death occurs without any history of CVD. These individuals may have only one, or none, of the traditional risk factors. Thus, novel biomarkers approaches may be necessary to supplement traditional risk factors in CVD diagnosis and prognosis. Early detection and frequent monitoring of both traditional risk factors and novel biomarkers has the potential to save lives and reduce unnecessary costs due to CVD morbidity and mortality.

To aid in disease identification and patient monitoring, clinical decision support systems (CDSSs) are being increasingly adopted to provide clinicians with personalized assessments or recommendations to assist in medical decisions. A popular topic in expert systems and AI in medicine, a CDSS is defined as “any electronic system which aids in the clinical decision making process in which data from individual patients are used to generate personalized assessments or recommendations that are then presented to clinicians for consideration” [90]. The CDSSs have several advantages relative to standard of care including the potential for faster diagnosis, improved prediction performance, and reduced medical costs via elimination of unnecessary testing. However, clinicians may be reluctant to adopt certain CDSSs which are based on “black box”
methods and provide results that are not easily interpreted in a clinical context. Likewise, lack of seamless integration between biomarker measurements and disease predictions may serve as a barrier to the broad-scale uptake of CDSS technologies. In an attempt to bridge the gap, this Chapter presents a CDSS for the prediction of a spectrum of CVD called the Cardiac ScoreCard. Based on a lasso logistic regression approach, the Cardiac ScoreCard algorithms combine patient demographics and novel protein biomarker data to form a single-valued “cardiac score” and clinically interpretable logistic regression coefficients. When fully developed, the Cardiac ScoreCard is intended to provide individualized assessments of cardiac health that are seamlessly integrated with biomarker measurements from POC medical microdevices [40].

When fully developed, mass produced, and validated clinically, LOC systems have the potential to simplify lab analysis routines, reduce sample and reagent volumes, shorten analysis times, and lower the cost of healthcare. There is a strong need for these medical microdevices that are both cost-effective and analytically robust. The p-BNC is a flexible detection platform that rivals established remote laboratory methods [38, 40]. Recent work has demonstrated the ‘macro’ laboratory based p-BNC’s multiplexed analyses of diverse analyte classes across several disease applications [24, 36, 49], for example, in the areas of HIV immune function [73], cardiac heart disease [27, 41, 91-93], ovarian cancer [37, 94], oral cancer [95, 96], prostate cancer, and the detection of drugs of abuse [39, 97]. While the p-BNC sensor platform offers a solution to the acquisition of cardiac biomarker measurements, the scope of this Chapter primarily covers the methods of converting biomarker and risk factor data streams into clinically usable and interpretable results. The various Cardiac ScoreCard models are intended to be used in
conjunction with the p-BNC, but they may act as standalone calculators when provided the necessary input parameters.

The objective of this work is to develop predictive models for a spectrum of CVD and do so in a manner that can be integrated seamlessly with multi-parameter biomarker measurements. This Chapter describes the process of developing the Cardiac ScoreCard and summarize the initial performance characteristics observed for two areas of cardiac disease, that is i) cardiac wellness and ii) HF diagnosis. The remainder of this Chapter is organized as follows. Section 4.3 reviews the literature for work related to CVD prediction models. Section 4.4 introduces the Cardiac ScoreCard approach and the role of biomarkers in CVD prediction. Descriptions of the clinical study data and the lasso logistic regression methods are provided in Section 4.5. Results and discussion for the cardiac wellness and HF models are covered in Section 4.6. Lastly, Section 4.7 concludes the Chapter with a brief summary of the models, their significance, and future directions.

4.3. Related work

4.3.1. Literature review

Clinical decision support tools are powerful expert systems with the potential to provide faster diagnoses, improved prediction performance, and reduced medical costs by eliminating unnecessary testing. Several CDSSs and related prediction models for the identification of heart diseases have been developed over the years, implementing a variety of techniques. One of the most commonly used techniques is artificial neural networks (ANN) due to its superior prediction performance and ability to identify
complex nonlinear patterns in the data [98]. In one of the first implementations for heart
disease applications, an ANN was trained on chest pain patients presenting to the
emergency room for the diagnosis of AMI [99]. Similarly, Furlong, Dupuy, & Heinsimer
developed an ANN for diagnosing AMI using serial cardiac enzyme data [100]. Yan,
Jiang, Zheng, Peng, & Li implemented a multilayer perceptron-based model that can
differentially diagnose five different cardiac outcomes [101]. Mehrabi, Maghsoudloo,
Arabalibeik, Noormand, & Nozari compared multilayer perceptron and radial basis
function neural networks for the discrimination of HF and COPD in 266 patients and 42
clinical variables [102].

Aside from neural networks, various other techniques have been successfully
implemented. Conforti & Guido used a Support vector machine (SVM) approach to
classify AMI and non-case patients [103]. Ion Titapiccolo et al. supported the use of
random forest models in predicting cardiovascular outcomes in hemodialysis patients due
to their ability to learn non-linear patterns in the feature space [104]. Vila-Francés et al.
employed a Bayesian network for the prediction of unstable angina (UA) [105]. Bayesian
models are attractive for clinical use because the relationships among variables are
represented by a graph and are, thus, easily interpreted by clinicians. Ensemble methods
are a useful strategy for increasing the generalization performance by combining the
posterior probabilities or predicted values from several base learners. Das, Turkoglu, &
Sengur improved prediction performance using an ANN ensemble method for heart
disease diagnosis on the Cleveland heart disease database [106]. A. Wang, An, Chen, Li,
& Alterovitz developed a low-cost and non-invasive screening system for hypertension
based on a hybrid logistic regression and an ANN model using only simple demographic data from questionnaire responses [107].

Feature selection is an important step in developing predictive models that eliminates irrelevant or redundant input parameters, resulting in reduced model complexity and improved generalization ability. Various feature selection methods have been attempted within the context of diagnosing heart diseases. For example, Nahar, Imam, Tickle, & Chen identified several key risk factors which contribute to heart disease using an association rule mining approach [108]. Shilaskar & Ghatol implemented a hybrid forward selection technique and SVM classifier which improved classification accuracy of various CVDs [109]. Shi et al. combined various feature selection approaches to identify a small subset of proteins and metabolites for the diagnosis of UA [110].

There are multiple scoring systems that profile CVD progression that have been widely adopted for risk estimation and clinical decision making. The Framingham 10-year CVD risk score [111] and Reynolds score [112, 113] predict CVD risk. A multi-parameter model with carotid intima-media thickness, presence of plaque, and traditional risk factors improved coronary heart disease risk prediction in the Atherosclerosis Risk in Communities (ARIC) study [114]. Recently, the Pooled Cohort Risk Equations expand utility and improve generalizability by combining several large cohort studies and is currently recommended for estimating 10-year risk for a first atherosclerotic CVD event [115]. The Thrombolysis in Myocardial Infarction (TIMI) risk score [116] estimates mortality for patients with UA and non-ST elevation myocardial infarction (NSTEMI).
The Seattle Heart Failure Model [117, 118] and Framingham profiles [119] estimate risk of congestive HF. Individually, these scores only provide narrow coverage across the broad spectrum of CVD progression. Additionally, many risk scores underutilize novel biomarker approaches, which, when combined with traditional risk factors, have been shown to boost prediction performance [120-122]. Despite the promise of increased performance, widespread adoption of multimarker assays and associated risk scores have not yet occurred, likely due to the absence of key technologies that seamlessly integrate biomarker measurements with disease prediction algorithms.

Some CDSSs for heart diseases have been applied to unique datasets with the inclusion of both patient demographic information and novel cardiac biomarker measurements. Notably, Eom, Kim, & Zhang performed differential heart disease diagnosis of normal, stable angina, UA, and AMI based on aptamer chip data using an ensemble with SVMs, neural networks, decision trees, and Bayesian networks [123]. Nambi et al. combined troponin T and NT-proBNP with age and race in a gender-specific model to improve HF prediction performance in the ARIC study [124]. Similarly, Blankenberg et al. used a lasso logistic regression approach with 30 biomarkers and demographics for 10-year cardiovascular risk estimation [121]. However, to our knowledge no previous studies have documented a multivariate index assay system for a spectrum of CVD that takes into account the practicality of parameter selection in building POC compatible multimarker panels. The bridge between model development/validation and integrated POC testing represents a key step in moving these promising theoretical approaches into mainstream clinical practice and is made possible via novel biosensing technologies, such as the recently developed p-BNC platform.
Compared to other CDSSs, the Cardiac ScoreCard system adds substantial value to clinicians and healthcare providers by streamlining patient sample collection, biomarker measurements, machine-learning algorithms, electronic medical records, and intuitive health report cards.

4.4. The Cardiac ScoreCard approach

Significant efforts over the past few decades have focused on translating biomarker assays to clinical practice. Most often, these efforts involve a long and uncertain pathway to discover, validate, and attain regulatory approval of a biomarker or panel of biomarkers for a specific application. However, this approach is enormously expensive and takes years to decades to complete [125]. Here a new cost- and time-effective pathway from discovery to clinical application is described through the development of the Cardiac ScoreCard. The Cardiac ScoreCard is an in vitro diagnostic multivariate index assay (IVDMIA), a device that uses multiple variables in an interpretation function to yield a single patient-specific result intended to diagnose, treat, or prevent disease and provides a result which cannot be independently derived by the end user [126]. The ScoreCard features a multiplexed panel of 14 cardiac biomarkers that can be applied in many ways depending on setting, symptoms, and medical history (Figure 4.1). Using measurements from a single chip format for multiple disease applications has the potential to significantly reduce time and costs associated with assay development and validation. This approach of making one chip for multiple applications leverages one of the key features of microelectronics whereby enormous scalability
advantages are secured by using standardized fabrication strategies and chip architectures with capacity to scale. In the clinical diagnostics area, developing a broad range of capabilities through the creation of a platform that can service multiple related applications has potential to streamline the development and regulatory approval process. This holistic diagnostic tool development strategy described here underlies what may be a new paradigm in multi-parameter clinical diagnostics. It is in this capacity that the ScoreCard’s predictive algorithms encompass three cardiovascular states: cardiac wellness, AMI, and HF.

Figure 4.1. The Cardiac ScoreCard uses biomarker measurements and traditional risk factors to make predictions and therapeutic recommendations for a spectrum of CVD. In this study, biomarker measurements were completed using standard methods including ELISA, LUMINEX, and Beckman Coulter Access; however, future measurements will
be completed using the p-BNC (top figure). The choice of Cardiac ScoreCard model largely depends on the context of the test based on the individual’s setting, symptoms, and medical history. This study describes the development of Cardiac ScoreCard algorithms for cardiac wellness and HF diagnosis, while AMI diagnosis and prognostic models for HF and AMI will be covered in future publications.

Currently, cardiac wellness testing relies in part on lipid profiling; however, nearly 50% of all heart attacks and strokes occur in patients with normal lipid levels [127]. Thus, there is need for a multimarker approach to risk profiling by supplementing lipid measurements with indicators of inflammation and plaque instability. The ScoreCard not only identifies high versus low risk individuals, but also provides a Cardiac Score that is indicative of overall cardiac health status. This wellness ScoreCard has potential in the future to be used by individuals in many settings such as pharmacies, clinics, workplaces, and homes. The ultimate goal of the cardiac wellness ScoreCard is CVD prevention by empowering individuals with a new tool to take control of their own heart health. Another advantageous feature of the wellness ScoreCard is that it uses the same panel as AMI (to be described in future publications) and HF ScoreCards, effectively creating an archive of baseline measurements for biomarkers of myocardial injury and hemodynamic stress.

Current guidelines recommend rapid response time in diagnosing AMI such that results are available as soon as possible, ideally within 60 minutes [128]. However, long delays in diagnosis often occur due to inefficiencies in central laboratory infrastructure. The AMI ScoreCard and the p-BNC have the potential to serve as a rapid POC device that can diagnose AMI at the patient bedside within minutes. The AMI ScoreCard may
also be used in ambulances, low-resource settings, and rural communities where timely diagnoses may significantly improve survival rates. Similarly, early and accurate diagnoses may improve outcomes for patients with HF [129]. The condition of HF may be difficult to diagnose due to the presentation of non-specific symptoms. Furthermore, the differential diagnosis of HF often requires numerous expensive procedures to obtain surrogate measures of cardiac function. However, natriuretic peptides such as brain natriuretic peptide (BNP) or NT-proBNP assays have shown significant potential for detecting, monitoring, and even guiding treatment [130]. Although models for AMI diagnosis, AMI prognosis, and HF prognosis are possible with these ScoreCard approaches, they are out of scope for the current study.

4.4.1. Biomarkers of CVD

Evidence has shown that employing a multimarker strategy with a pathobiologically diverse set of biomarkers adds substantial predictive information in the risk assessment of ACS [131, 132]. Likewise, by selecting a panel of biomarkers that are differentially expressed across all stages of CVD progression through different pathways, there may be opportunities to improve risk prediction. Also important is the relationship between biomarker levels. In general, a few uncorrelated predictors will perform better than many strongly correlated predictors. Since biomarkers from the same pathophysiology are often correlated with each other, it is advantageous to use uncorrelated biomarkers expressed in various stages of CVD through different pathophysiological pathways. Figure 4.2 shows the biomarkers in this study grouped by their known CVD pathophysiology [133] and relationships via Pearson correlation.
coefficient matrix generated using data from the current study, as described in Section 4.5. As plaque begins to form, mature, destabilize, and rupture, leading to the manifestation of ischemia, thrombosis, myocardial damage, and subsequent myocardial remodeling, certain cardiac biomarkers are differentially expressed [134]. The relative expression of these biomarkers forms a signature that may be used to pinpoint the status of an individual’s CVD pathophysiology.

It is important to note that some cardiac biomarkers are not specific to a single pathway, but may be expressed in multiple CVD pathways. For example, BNP is a marker of hemodynamic stress that is released during ischemia and often monitored in patients with HF; however, it is also elevated upon ventricular wall stress and may be used as a predictor for other vascular events. Moreover, BNP is secreted almost exclusively from the heart and is thus more sensitive than non-specific markers that are secreted from other tissues [135]. Similarly, cardiac troponins, first recognized as biomarkers for AMI [136], are the standard biomarkers for diagnosis of myocardial damage. However, recent developments in ultra-high sensitivity assay technology show that low levels of elevated troponin yield prognostic information for individuals without known CVD [137-140]. The information encoded in biomarkers from various CVD pathways has enormous potential for diagnostic and prognostic applications.
Figure 4.2. Cardiac biomarkers grouped by known CVD pathophysiology and Pearson correlation coefficient matrix. An idealized diagram (left) shows stages of atherosclerotic plaque development and associated biomarkers. A correlation matrix (right) shows the Pearson correlation coefficient matrix of evaluable data for all patients (N = 579). Correlations are derived from log-normalized, standardized, and imputed median serum biomarker concentrations across all time points for all patient outcomes in the current study. Details of the clinical studies and data handling procedures are described in Section 4.5.

4.5. Data and methods

4.5.1. Logistic regression

Logistic regression is well-suited for use in diagnostic models. For one, logistic regression allows the analysis of dichotomous outcomes with two mutually exclusive levels, e.g., disease and non-case, and the logistic regression is regarded as the standard method of analysis for this situation [141, 142]. Additionally, unlike most other
classification models in statistical learning, logistic regression is probabilistic and is thus more intuitive than its non-probabilistic counterparts. Both the logistic regression’s interpretability and ability to function as a dichotomous classifier has given rise to its widespread popularity in the clinical community. Logistic regression models are fit using maximum likelihood given by Equation 4.1.

$$\max_{\beta, \beta_0} \left\{ \sum_{i=1}^{N} \left[ y_i (\beta_0 + \beta^T x_i) - \log(1 + e^{\beta_0 + \beta^T x_i}) \right] \right\}$$

Equation 4.1. Maximum likelihood for the logistic regression.

Here, $x_i$ are the inputs, $y_i$ are the responses, and $\beta$ are the coefficients [143]. In the following section, a lasso penalty is applied to the logistic regression to perform model shrinkage and selection.

**4.5.2. Lasso penalty for model shrinkage and selection**

When there are many correlated parameters in a logistic regression model, their coefficients may exhibit high variance. Imposing a size constraint, or shrinkage, on the coefficients alleviates this problem. The lasso penalty is a method for performing shrinkage that was originally introduced for use in least squares regression [144]; however, it may be applied to other methods such as the logistic regression. Perhaps the most notable property of the lasso is its ability to perform automatic parameter selection. The goal of parameter selection is to retain a subset of the relevant predictors and discard the rest. Alternative methods perform subset selection in a stepwise fashion, which is a
discrete process (i.e., variables are either retained or discarded), and it often exhibits high variance and does not reduce prediction error of the full model [145]. On the other hand, shrinkage methods like the lasso are more continuous and do not suffer as much from high variability [143]. The lasso method makes a size constraint on parameters subject to the L_1 lasso penalty, \( \sum_{j=1}^{p} |\beta_j| \), and the log-likelihood takes the form of Equation 4.2.

\[
\max_{\beta, \beta_0} \left\{ \sum_{i=1}^{N} \left[ y_i (\beta_0 + \beta^T x_i) - \log\left(1 + e^{\beta_0 + \beta^T x_i}\right)\right] - \lambda \sum_{j=1}^{p} |\beta_j| \right\}
\]

Equation 4.2. Maximum likelihood for the lasso logistic regression.

Here, \( \lambda \) is the complexity parameter that controls amount of shrinkage. Cross-validation is used to adaptively select an appropriate \( \lambda \) that minimizes the deviance. Figure 4.3 demonstrates this concept through the L_1 regularization path for HF diagnosis as an example. The coefficients, \( \beta_j \), are evaluated for each parameter as \( \lambda \) is varied between 0 and 1. When \( \lambda = 0 \), the coefficients are simply those given by logistic regression (Equation 4.1). However, as \( \lambda \) increases, the coefficients shrink towards zero and towards each other. Increasing \( \lambda \) may also make some coefficients exactly zero; thus, the lasso performs a kind of continuous subset selection that is preferable over stepwise methods. Note that the intercept term \( \beta_0 \) is excluded from the L_1 penalty because penalization of the intercept would introduce bias dependent on the origin of \( y \) when selecting the penalty term. The cross-validated \( \lambda \) that minimizes the deviance of the test set is often selected for the final model; however, \( \lambda \) one standard error (SE) above the
minimum deviance may be used to obtain a more sparse solution and to avoid the potential effects of an asymptotic deviance at low values of \( \lambda \).

Figure 4.3. Trace plot of coefficients fit by lasso logistic regression for HF diagnosis. When \( \lambda \) is close to 0, the ordinary logistic regression estimates emerge. As \( \lambda \) increases, the coefficients shrink towards zero and towards each other.

4.5.3. Description of clinical studies

The Cardiac ScoreCard models were developed using data merged from two clinical studies. A cross-sectional biomarker discovery study at the University of Kentucky (UK) previously described [27, 93] identified 13 relevant biomarkers for screening AMI. Serum and salivary samples were collected from AMI patients \((N = 90)\) 0 to 48 hours from presentation to the emergency department and recruited healthy controls.
The primary outcomes of this study are ST segment elevation MI (STEMI), NSTEMI, and control. Biomarkers measured include cTnI, CK-MB, CRP, myeloperoxidase (MPO), MYO, BNP, adiponectin, CD40 ligand (CD40L), interleukin-1 beta (IL-1β), matrix metalloproteinase 9 (MMP-9), regulated on activation normal T cell expressed and presumably secreted (RANTES), soluble intracellular adhesion molecule 1 (sICAM-1), and tumor necrosis factor alpha (TNF-α). These data were supplemented with preliminary data from a similar clinical study focusing on AMI diagnosis in the Texas Medical Center (TMC) in Houston, TX (clinical finding results to be published separately). Patients who presented to the ED with chest pain or AMI related symptoms \( (N = 389) \) and at least 21 years of age were enrolled. Serum samples were collected at 1, 3, 6, and 12 hours after presentation to the ED, and samples for BNP were collected separately and analyzed at the clinical site immediately after collection. Samples were assayed for the same 13 cardiac biomarkers and D-dimer via gold standard methods \( (e.g., \; \text{ELISA, LUMINEX, and Beckman Coulter Access}) \). The outcomes of this study were ACS comprising STEMI, NSTEMI, and UA, indeterminate, unknown, non-case chest pain, congestive HF, and chronic kidney disease (CKD). The distributions of patient outcomes from the merged clinical studies are summarized in Table 4.1.
Table 4.1. Number and percent of total patient outcomes (N = 579) from merged clinical study data.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>100 (17)</td>
</tr>
<tr>
<td>Non-case chest pain</td>
<td>182 (31)</td>
</tr>
<tr>
<td>HF</td>
<td>81 (14)</td>
</tr>
<tr>
<td>ACS</td>
<td>145 (25)</td>
</tr>
<tr>
<td>UA</td>
<td>31 (5)</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>48 (8)</td>
</tr>
<tr>
<td>STEMI</td>
<td>66 (11)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>17 (3)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (1)</td>
</tr>
<tr>
<td>CKD</td>
<td>50 (9)</td>
</tr>
</tbody>
</table>

4.5.4. Cardiac wellness

Figure 4.4 summarizes the flow of patient data for the development of the cardiac wellness model. A lasso logistic regression model was developed using data from two clinical studies, UK (N = 100) and TMC (N = 389). Here, only recruited healthy controls were included from the UK study whereas no healthy patients were enrolled in the TMC clinical study. Patients from UK presenting with UA, HF, CKD, indeterminate, or unknown status (N = 207) were excluded from the analysis. Patients with substantial data missing (> 50%) across the evaluated parameters were also excluded (N = 33). The remaining patients (N = 249) were dichotomized into two classifications, high risk (N = 154) and low risk (N = 95), according to the presence of morbidities associated with CVD risk described as follows.

One of the challenges in developing a model for cardiac wellness is the difficulty of obtaining relevant clinical endpoints which directly reflect health status. Long-term
prospective studies like the Framingham Heart Study are particularly well-suited for gathering hard clinical endpoints such as morbidity and mortality; however, these studies are enormously expensive and take decades to complete. Here, to make immediate progress towards new model development, a risk stratification approach was used to develop a model of overall cardiac wellness. Several approaches to grouping patients by risk have been explored, such as counting risk factors for persons without known CVD or diabetes [146] and grouping patients according to Framingham scores for low risk (< 6% in 10 years), medium risk (6-20% in 10 years), and high risk (> 20% in 10 years) [147]. Here, subjects were classified into low and high risk groups using the presence of hyperlipidemia, hypertension, stroke, diabetes, previous MI, and known CAD as risk factors. Patients with one or more risk factors were categorized as high risk for CVD while patients with zero risk factors were categorized as low risk. A logistic regression model with lasso penalty was trained using risk determination and a total of 18 parameters including age, gender, body mass index (BMI), smoking, and 14 cardiac biomarker measurements. Since the patient outcomes were defined by the number of risk factors present, it was necessary to exclude these factors as parameters in the model to avoid overfitting. Therefore, cholesterol, blood pressure, and diabetes were not included in the model parameters.
4.5.5. HF diagnosis

The development of the HF model follows a similar procedure. Figure 4.5 reveals the flow of patient data for development of the HF diagnostic model. Again, a lasso logistic regression model was developed using data from two clinical studies, UK (N = 190) and TMC (N = 389). Patients presenting with UA, NSTEMI, STEMI, indeterminate, or unknown status (N = 171) were excluded from the analysis. Patients with substantial data missing (> 50%) across the evaluated parameters were also excluded (N = 52). The remaining patients (N = 356) were dichotomized into two classifications, HF (N = 83)
and non-case ($N = 273$), according to history of HF or HF at admission. A total of 19 parameters were considered in this HF diagnostic model including diabetes, hypertension, gender, BMI, age, and 14 cardiac biomarker measurements.

![Image](image.png)

Figure 4.5. HF model data flow and inclusion criteria.

### 4.5.6. Data standardization and imputation

For both models continuous parameters, not including age and BMI, were transformed with natural log. Then, all data were standardized with zero mean and unit variance. Biomarker measurements that were below LOD were reassigned to their respective LOD, and biomarker concentrations recorded above assay range were set to
the assay upper limit. Median biomarker concentrations across 1, 3, 6, and 12 hour time points were used for the models.

Missing data were imputed with a multiple imputation procedure in statistical software R using the multivariate imputation by chained equations (MICE) algorithm [148]. Multiple imputation is the preferred method for solving complex incomplete data problems [148, 149], and it attempts to simulate the process that created the missing data and preserve relations in the data. Uncertainty regarding the relations between missing data are accounted for in the magnitude of differences between imputed data sets. The imputation procedure was performed in three main steps. For the purpose of this study, the data were assumed missing at random. First, a total of ten imputations were generated using predictive mean matching and logistic regression imputation models for numeric and categorical data, respectively. Then, model training and selection were performed on each individual imputed data set. Lastly, the performance of the trained models, including area under the receiver operating characteristic (ROC) curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were evaluated for pooled Cardiac ScoreCard estimates.

4.5.7. Model training and selection

The same model training and selection procedure was performed for cardiac wellness and HF models. The data were first partitioned using stratified 5-fold cross-validation, preserving the relative proportions of disease outcomes across each fold, with four folds for training and one fold for testing. For each iteration of cross-validation, models were trained for each $m = 10$ imputed datasets. Models were trained by fitting
lasso penalized logistic regression coefficients using 50 log-linearly spaced $\lambda$ values between 0 and 1. Deviance was estimated using 5-fold cross-validation within the training set, separating the data into four folds for the partial training set and one fold for the validation set. Models were selected for $\lambda$ that minimized deviance of the validation set (for cardiac wellness model) or for $\lambda$ one SE above the minimum deviance (for HF model). The selected model was then retrained using the full training set, and the training error and test error were evaluated for each imputed dataset. The logistic regression probability, or “score”, was evaluated for the test set. The mean scores of the imputed datasets were evaluated for each fold. The mean score across each fold was evaluated, and predicted class labels were defined as “case” if the corresponding score was greater than the optimal cutoff given by ROC analysis.

Models were internally validated with 5-fold cross-validation, and performance was measured by AUC, sensitivity, specificity, PPV, and NPV. Discrimination improvement was determined by measuring difference in AUC of the ScoreCard and various reference methods using a one-sided test of significance with $p = 0.05$ [150]. The cardiac wellness model discrimination was compared against the Framingham 10-year CVD risk score and a model comprising only the 14 biomarker panel. The Framingham risk score was calculated for each patient using methods described previously [111], and the risk predictions were informed by the ROC’s optimal cutoff value. A lasso logistic regression model comprised of the 14 biomarkers was developed using the same approach as described. Cardiac wellness model calibration was performed by sorting patients into deciles of risk, and the Hosmer-Lemeshow goodness of fit statistic was used to determine whether the model adequately fits the data. The HF diagnosis model
performance was compared with BNP, and the optimal cutoff from ROC analysis was used (67.0 pg/mL).

Hereafter, each model is referred as a “ScoreCard” with the outcome of each model, or probability, denoted as the “Cardiac Score”. For the cardiac wellness model, Cardiac Score is treated as an indicator for overall cardiac wellness status, whereas in the HF model, Cardiac Score is used to generate disease predictions for diagnostic purposes and is evaluated as such. The difference in scoring procedure here employed reflects the anticipated end use criteria whereby the wellness model is expected to be used by the consumer wellness community and the HF model is projected to be used more directly by the clinical community.

4.6. Results and discussion

4.6.1. Cardiac wellness results

A lasso logistic regression model for the prediction of cardiac wellness was developed. Figure 4.6A shows ROC curves for Framingham 10-year CVD risk score, biomarker model, and the ScoreCard. The performance of each model is summarized in Table 4.2. The Framingham risk score shows satisfactory discrimination between risk groups (AUC = 0.7975 [95% CI, 0.7431, 0.8518]). The ScoreCard (AUC = 0.8403 [95% CI, 0.7924, 0.8881]) increases AUC over Framingham by 0.0428 (p = 0.1222). Biomarkers alone exhibited slightly worse discrimination than Framingham and the ScoreCard (AUC = 0.7664 [95% CI, 0.7087, 0.8241]). The ScoreCard significantly improves AUC over the biomarker model by 0.0739 (p = 0.0260), demonstrating the
importance of traditional risk factors in new biomarker models. Figure 4.6B shows good calibration of Cardiac Score with observed proportions of high risk patients across deciles of predicted risk. A non-significant result of the Hosmer-Lemeshow goodness of fit test suggests there is no evidence of a poor fit ($p = 0.9754$).

Out of the 18 input parameters, the lasso penalty selected 15 (given by nonzero coefficient) with BMI ($\beta_{BMI} = 0.8243$), smoking ($\beta_{Smoking} = 0.4711$), age ($\beta_{Age} = 0.4457$), serum myoglobin ($\beta_{MYO} = 0.3440$), gender ($\beta_{Gender} = 0.1939$), and serum IL-1$\beta$ ($\beta_{IL-1\beta} = 0.1689$) having the largest effect sizes (Table 4.3). These results suggest that, in addition to the important traditional risk factors, multiple biomarkers representing a diverse pathophysiology are necessary for assessing and predicting cardiac wellness.

The combination of multiple biomarkers and selected traditional risk factors into a multivariate index assay shows strong potential for discriminating high risk and low risk patients. However, one limitation in this study is that the models were trained using samples collected in the context of cardiac events. Thus, these models may need to be optimized further to service scenarios where preventative measurements are taken prior to an event. Also, this comparison of the Cardiac ScoreCard with the Framingham Risk Score is biased as the number of risk factors (presence of hyperlipidemia, hypertension, stroke, diabetes, previous MI, and known CAD) determined the high and low risk categories. Nonetheless, the acquisition of data using these convenient samples has provided quicker access to the initial models here presented.
Table 4.2. Summary of performance for Framingham 10-year CVD risk, a model with only biomarkers, and Wellness ScoreCard. Sensitivity, specificity, PPV, and NPV (95% confidence intervals) are determined by optimal cutoff given by ROC analysis.

<table>
<thead>
<tr>
<th></th>
<th>Framingham</th>
<th>Biomarkers</th>
<th>Wellness ScoreCard</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC</strong></td>
<td>0.7975</td>
<td>0.7664</td>
<td>0.8403</td>
</tr>
<tr>
<td></td>
<td>(0.7431 - 0.8518)</td>
<td>(0.7087 - 0.8241)</td>
<td>(0.7924 - 0.8881)</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>0.7403</td>
<td>0.6948</td>
<td>0.7597</td>
</tr>
<tr>
<td></td>
<td>(0.6803 - 0.7929)</td>
<td>(0.6329 - 0.7508)</td>
<td>(0.7009 - 0.8107)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>0.7474</td>
<td>0.7474</td>
<td>0.8211</td>
</tr>
<tr>
<td></td>
<td>(0.6878 - 0.7994)</td>
<td>(0.6878 - 0.7994)</td>
<td>(0.7665 - 0.8659)</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>0.8261</td>
<td>0.8168</td>
<td>0.8731</td>
</tr>
<tr>
<td></td>
<td>(0.7720 - 0.8703)</td>
<td>(0.7619 - 0.8621)</td>
<td>(0.8238 - 0.9111)</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>0.6396</td>
<td>0.6017</td>
<td>0.6783</td>
</tr>
<tr>
<td></td>
<td>(0.5763 - 0.6988)</td>
<td>(0.5378 - 0.6625)</td>
<td>(0.6158 - 0.7353)</td>
</tr>
<tr>
<td><strong>Training error</strong></td>
<td>-</td>
<td>-</td>
<td>0.2112</td>
</tr>
<tr>
<td><strong>Test error</strong></td>
<td>-</td>
<td>-</td>
<td>0.2429</td>
</tr>
</tbody>
</table>
Table 4.3. Lasso logistic regression coefficients for Wellness and HF ScoreCards.

<table>
<thead>
<tr>
<th></th>
<th>Wellness ScoreCard</th>
<th>HF ScoreCard</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$ (intercept)</td>
<td>-0.0081</td>
<td>-1.6448</td>
</tr>
<tr>
<td>$\beta_{cTnl}$</td>
<td>0.0986</td>
<td>0.2794</td>
</tr>
<tr>
<td>$\beta_{CK-MB}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{CRP}$</td>
<td>0.1450</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{MPO}$</td>
<td>0.0026</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{MYO}$</td>
<td>0.3440</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{BNP}$</td>
<td>0.1046</td>
<td>1.5120</td>
</tr>
<tr>
<td>$\beta_{Adiponectin}$</td>
<td>-0.0626</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{CD40L}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{IL-1\beta}$</td>
<td>0.1689</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{MMP-9}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{RANTES}$</td>
<td>-0.1021</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{sICAM-1}$</td>
<td>-0.1642</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{TNF-\alpha}$</td>
<td>-0.0225</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{D-dimer}$</td>
<td>0.0775</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{Age}$</td>
<td>0.4457</td>
<td>0.0610</td>
</tr>
<tr>
<td>$\beta_{BMI}$</td>
<td>0.8243</td>
<td>0.2485</td>
</tr>
<tr>
<td>$\beta_{Gender}$</td>
<td>0.1939</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{Smoking}$</td>
<td>0.4711</td>
<td>*</td>
</tr>
<tr>
<td>$\beta_{Hypertension}$</td>
<td>*</td>
<td>0</td>
</tr>
<tr>
<td>$\beta_{Diabetes}$</td>
<td>*</td>
<td>0</td>
</tr>
</tbody>
</table>

* Parameter excluded from the model
Figure 4.6. Cardiac wellness model discrimination and calibration. (A) The ROC curves for Framingham 10-year CVD risk score, a biomarker model, and the Cardiac ScoreCard. (B) Plot showing calibration for the wellness ScoreCard for patients sorted by deciles of predicted risk. Both discrimination and calibration results were generated using median diagnosis predictions and mean ScoreCard values across 10 imputations from stratified 5-fold cross-validated test sets.

4.6.2. HF diagnosis results

A lasso logistic regression model for the diagnosis of HF was developed. Figure 4.7 shows ROC curves for BNP and Cardiac ScoreCard, and the performances of the ScoreCard and BNP are summarized in Table 4.4. The BNP values show adequate discrimination in diagnosing HF (AUC = 0.9320 [95% CI, 0.8936, 0.9704]). The ScoreCard (AUC = 0.9412 [95% CI, 0.9053, 0.9771]) shows slight improvements in AUC by 0.0092 ($p = 0.3659$).
Table 4.4. Summary of performance for HF ScoreCard versus BNP. Sensitivity, specificity, PPV, and NPV (95% confidence intervals) are determined by optimal cutoff given by ROC analysis.

<table>
<thead>
<tr>
<th></th>
<th>BNP</th>
<th>HF ScoreCard</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>0.9320 (0.8936 – 0.9704)</td>
<td>0.9412 (0.9053 - 0.9771)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.8313 (0.7871 - 0.8684)</td>
<td>0.8554 (0.8133 - 0.8899)</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.8959 (0.8580 - 0.9252)</td>
<td>0.9145 (0.8790 - 0.9410)</td>
</tr>
<tr>
<td>PPV</td>
<td>0.7113 (0.6604 - 0.7577)</td>
<td>0.7553 (0.7063 - 0.7988)</td>
</tr>
<tr>
<td>NPV</td>
<td>0.9451 (0.9145 - 0.9661)</td>
<td>0.9535 (0.9245 - 0.9727)</td>
</tr>
<tr>
<td>Training error</td>
<td>-</td>
<td>0.1614</td>
</tr>
<tr>
<td>Test error</td>
<td>-</td>
<td>0.1810</td>
</tr>
</tbody>
</table>

Figure 4.7. The ROC curves for BNP and the HF ScoreCard. Results were generated using median diagnosis predictions across 10 imputations from stratified 5-fold cross-validated test sets.

This lasso-based parameter selection approach resulted in a sparse model with four non-zero parameters: BNP ($\beta_{BNP} = 1.5120$), cTnI ($\beta_{cTnI} = 0.2794$), BMI ($\beta_{BMI} =$
0.2485), and age ($\beta_{Age} = 0.0610$) with all other parameter coefficients having no effect on the response (Table 4.3). As expected from the literature, BNP is the strongest indicator for presence of HF. From a practical standpoint, this sparse model of HF diagnosis prioritizes two out of the original 14 biomarkers, thus reducing costs and minimizing assay complexity.

Although improvements in discrimination were non-significant, the ScoreCard demonstrates its utility as a diagnostic indicator for HF. Here, sensitivity, specificity, PPV, and NPV are given for the optimal ROC cutoff, but the ScoreCard may be tuned for ruling in and ruling out HF in various clinical decision scenarios.

### 4.7. Conclusions

In this Chapter, a CDSS for the diagnosis and assessment of a spectrum of CVD, the Cardiac ScoreCard, was presented. There are several contributions that distinguish this approach from previous work related to expert systems. (1) Patient demographics and novel biomarker measurements from a diverse pathophysiology were combined to predict multiple CVD outcomes. One of the strengths of logistic regression is its ability to predict the presence of a disease based on independent variables which are continuous, categorical, or both. These results suggest that augmenting the traditional risk factors (categorical) with a multimarker panel of protein biomarkers (continuous) provides improved performance over reference methods in both HF and wellness prediction. (2) To overcome issues with interpretability in CDSSs, we provide not only disease predictions, but also clinical insights in the form of logistic regression coefficients.
Although other CDSSs that use “black box” methods (e.g., ANNs and SVMs) often outperform other more basic learners in terms of classification performance, they are not easily interpreted by clinicians because of their non-linear weights and feature mappings. In general, these methods do not provide interpretable rationale for diagnostic decisions, nor do they provide the requisite information needed to make patient-specific recommendations for treatment. (3) We provide a framework for the integration with POC medical microdevices. The Cardiac ScoreCard leverages a single biomarker panel for multiple CVD applications. Consequently, this approach has the potential to greatly reduce costs and time associated with assay development, validation, and approval. While many CDSSs reported previously are largely decoupled from the data source, the Cardiac ScoreCard is intricately linked to the clinical testing method [40] and, as a result, may provide substantial added value to healthcare providers. Furthermore, the lasso-based parameter selection approach used in this study plays an important role in the down-selection of relevant biomarkers. In general, input variables should be highly correlated with the response, but uncorrelated with each other. Additionally, when developing a multiplexed immunoassay it is critically important to limit the total number of biomarkers assayed to a reasonable amount in order to alleviate the effects of non-specific binding and also to reduce cost associated with expensive antibody reagents. The lasso-based approach developed here plays a practical role in parameter reduction by selecting the relevant biomarkers for inclusion in multiplex panels.

Despite the unique contributions of the Cardiac ScoreCard approach, this study has several limitations. First, only a single classification technique was applied and compared to reference methods. As reported previously, the feature space of cardiac-
related data may contain nonlinear patterns that would otherwise be undetectable without the use of nonlinear classification methods [98]. In future studies, a variety of techniques (e.g., ANNs, SVMs, decision trees, Bayesian networks, logistic regression, etc.) should be compared side-by-side. In addition, ensembles combining these base learners should be developed to further improve model performance and generalization. Second, model performance was estimated here using cross-validation. Validation using an external and independent test set will be necessary to show model generalizability. Another limitation of this study was the need to combine two datasets in order to provide enough samples for case and non-case discrimination in HF and wellness models. Lastly, longitudinal studies based on the Cardiac ScoreCard are needed to examine the effect on patient outcomes and to determine personalized treatments. Albeit preliminary and in need of further validation, initial results suggest the utility of the Cardiac ScoreCard in predicting cardiac wellness and HF.

Despite various limitations, these initial studies highlight key recommendations for future efforts in the areas of CVD prediction and multivariate index assay development. When developing CDSSs for CVD, researchers should consider incorporating models, such as decision trees, Bayesian networks, or logistic regression, which are easier to interpret and may provide better diagnostic support information to clinicians. Prioritizing interpretability may help minimize the clinical community’s reluctance to adopt these new CDSSs. In addition, researchers should consider using practical parameter selection techniques when developing multiplexed immunoassays in order to retain a more manageable number of biomarkers, greatly simplify the assay chemistry, and reduce the cost of reagents. Lastly, researchers developing new algorithms
for medical uses should not underestimate the importance of using real clinical data from
the target population. While some publicly available datasets are perfect for testing new
techniques and comparing performances with previous efforts, better prediction
performance on these datasets does not necessarily generalize to future data. Thus,
models should be developed using real clinical data, acquired in a way that replicates its
targeted use, and validated using a similarly-derived independent test set.
The potent combination of medical microdevices, new biomarker measurements, and machine learning has the potential to transform medicine by empowering individuals to play more active roles in the management of their own wellness status. These marker-driven tests have the possibility to radically reduce costs, decrease wait times, and add new options for patients needing regular health monitoring. Further, these efforts demonstrate the clinical utility of fusing data from the information-rich IoB and IoT and use predictive analytics to generate consumer-friendly, single-index assessments for wellness/illness status. Tools of this nature provide new options for personalized wellness management and help to define the pathway to “exponential medicine”.

Looking to the future, we believe that the LOC/microfluidic and bioanalysis communities will increasingly rely on the generation of large datasets and successful implementation of machine-learning algorithms for unprecedented performance in
disease diagnosis and prognosis. While the race to acquire massive amounts of health information is in its infancy (e.g., initiated by large pharmaceutical/medical corporations, insurance companies, or governments), the applications of these high quality and high-dimensional clinical datasets will play a large role in medicine in the years to come as direct to consumer testing becomes more prominent. Thus, it is important for device developers to establish strong clinical collaborations to identify disease targets, oversee the generation of standardized testing protocols, and address unmet clinical needs such that the new LOC technology has the largest possible social and economic impact. Additionally, with the ever-increasing value and volume of data, we will be continually faced with ethical questions regarding the ownership and use of these sensitive data. Researchers developing new CDSSs need to prioritize patient confidentiality by designing systems that provide secure user authentication and data transactions.

In addition to privacy concerns, issues with the integration of data acquisition, handling, and interpretation continues to plague the healthcare industry. One of the main advantages of LOC devices is their high level of integration and automation; however, despite the fact that many clinical analyzers currently possess the requisite tools to acquire, process, and transmit data, it is not uncommon to see healthcare professionals manually transcribing data from one system to another because of inadequate design and the absence data exchange standards [151]. Integrated chem- and bio-sensor platform developers need to design universal systems that are compatible with the existing workflows of healthcare providers in order to streamline data transactions.
Lastly, clinicians may be reluctant to adopt CDSSs that use “black box” methods (e.g., ANNs and SVMs) which provide results that are not easily interpreted. When developing machine-learning algorithms, researchers should implement models that provide both interpretable rationale for diagnostic decisions and the essential information to make patient-specific recommendations. Further, researchers need to convey biomarker information more effectively to the general population, for example, by developing health report cards that convert biomarker measurements into intuitive wellness scores. Given the tools to track wellness over time, patients learn from their intimate connection with intuitive data streams and are able to play more active roles in managing their cardiac health.

With a significant portion of the infrastructure now in place, the capacity to learn may be accelerated. At the time of writing this dissertation, the devices described herein have moved to commercial partners for scaling. Additionally, a new Center has been established to gather significant quantities of data for the purpose of refining the Cardiac ScoreCard algorithms. Linkages to racially diverse clinical trials have also been established for the purpose of improving the level of validation for these cardiac risk calculators. This combination of a platform to digitize biology and predictive analytics has the potential to alter the trajectory of medicine, where the current linear thinking—mainly based on late-stage disease diagnosis using expensive and cumbersome tools—is replaced by a pathway to exponential medicine made possible through the introduction of scalable tools with the capacity to learn.
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