

Programmable Bio-nanochip Platform: A Point-of-Care Biosensor System with the Capacity To Learn

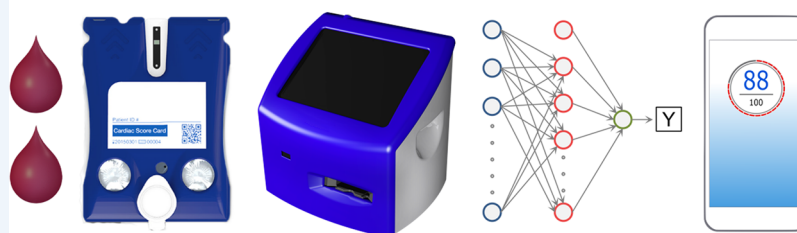
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Programmable Bio-Nano-Chips: Sensors That Learn



CONSPECTUS: The combination of point-of-care (POC) medical microdevices and machine learning has the potential transform the practice of medicine. In this area, scalable lab-on-a-chip (LOC) devices have many advantages over standard laboratory methods, including faster analysis, reduced cost, lower power consumption, and higher levels of integration and automation. Despite significant advances in LOC technologies over the years, several remaining obstacles are preventing clinical implementation and market penetration of these novel medical microdevices. Similarly, while machine learning has seen explosive growth in recent years and promises to shift the practice of medicine toward data-intensive and evidence-based decision making, its uptake has been hindered due to the lack of integration between clinical measurements and disease determinations. In this Account, we describe recent developments in the programmable bio-nanochip (p-BNC) system, a biosensor platform with the capacity for learning. The p-BNC is a “platform to digitize biology” in which small quantities of patient sample generate immunofluorescent signal on agarose bead sensors that is optically extracted and converted to antigen concentrations. The platform comprises disposable microfluidic cartridges, a portable analyzer, automated data analysis software, and intuitive mobile health interfaces. The single-use cartridges are fully integrated, self-contained microfluidic devices containing aqueous buffers conveniently embedded for POC use. A novel fluid delivery method was developed to provide accurate and repeatable flow rates via actuation of the cartridge’s blister packs. A portable analyzer instrument was designed to integrate fluid delivery, optical detection, image analysis, and user interface, representing a universal system for acquiring, processing, and managing clinical data while overcoming many of the challenges facing the widespread clinical adoption of LOC technologies. We demonstrate the p-BNC’s flexibility through the completion of multiplex assays within the single-use disposable cartridges for three clinical applications: prostate cancer, ovarian cancer, and acute myocardial infarction.

Toward the goal of creating “sensors that learn”, we have developed and describe here the Cardiac ScoreCard, a clinical decision support system for a spectrum of cardiovascular disease. The Cardiac ScoreCard approach comprises a comprehensive biomarker panel and risk factor information in a predictive model capable of assessing early risk and late-stage disease progression for heart attack and heart failure patients. These marker-driven tests have the potential to radically reduce costs, decrease wait times, and introduce new options for patients needing regular health monitoring. Further, these efforts demonstrate the clinical utility of fusing data from information-rich biomarkers and the Internet of Things (IoT) using predictive analytics to generate single-index assessments for wellness/illness status. By promoting disease prevention and personalized wellness management, tools of this nature have the potential to improve health care exponentially.

■ INTRODUCTION

We are entering a new era of chem- and biosensing 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 environ-

ment through the Internet of Things (IoT). Extending these transformative changes to health care has the potential to exponentially improve lives. Despite the ubiquity of physical silicon transducers in various mobile devices today, there is a

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lack of mobile health (mHealth) biomarker measurement platforms that are programmable (i.e., can be easily retasked for a variety of applications) and accessible to individuals, chemists, pharmaceutical scientists, and care-providers, alike. While nearly 70% of current medical decisions are made using diagnostic tests,¹ these tests for the most part are currently performed in traditional healthcare settings using phlebotomists, remote laboratories, delayed reporting, and an inefficient workflow that stifles the arrival of novel biosensor technologies with the capacity to transform clinical testing and medical decision making.

Microfluidic and lab-on-a-chip (LOC) systems are strong candidates for providing the necessary “hardware” for these chem- and biosensors. Originally inspired by microfabrication techniques from the microelectronics industry,^{2,3} LOC approaches have made their way into several applications since their introduction in the early 1990s,⁴ finding utility in medicine, inkjet printers, separation sciences, food safety, military, and veterinary markets. Arguably, point-of-care (POC) diagnostics is the most promising application for LOC technology, where scalable medical microdevices offer faster analysis times, reduced volumes of bioreagents, lower power requirements, and higher levels of integration and automation than standard central and remote laboratory methods.⁵ However, despite the potentially enormous societal impact of LOC technology, major barriers are preventing the translation of these novel systems from the laboratory to routine clinical practice, such as lack of integration and failure to compete with both performance and cost of laboratory-based tests.^{6–8} Further, the field is currently experiencing significant challenges associated with overly aggressive projections of current capabilities of selected efforts. Despite these challenges, there is great opportunity for LOC technologies provided that open and honest evaluations comprising extensive clinical validation and peer-reviewed reports are made widely available to clinical, regulatory, commercial, and general public audiences.

Similarly, machine learning has seen explosive growth in recent years due to the emergence of new data mining techniques, the increasing availability of data, and the decreasing cost of computation.⁹ This widespread adoption of artificial intelligence (AI) systems over the past two decades has resulted in a paradigm shift toward data-intensive and evidence-based decision making, spanning a variety of disciplines including chemistry across government, industry, and academic institutions, alike. Likewise, machine learning is playing an increasingly important role in chem- and biosensing applications and within the practice of medicine; however, despite enormous technological progress, several challenges are preventing AI systems from reaching their full potential.¹⁰ 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 AI biosensor systems to significantly improve health care by acquiring, processing, and managing clinical data.

In this Account, we describe our most recent work toward developing the programmable bio-nanochip (p-BNC) ensemble with the capacity to learn.^{11–16} This multiplex and multiclass 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.¹⁷ The p-BNC system

shown in Figure 1 is a flexible platform for digitizing biology, featuring sensor ensembles that measure biomarkers in a highly

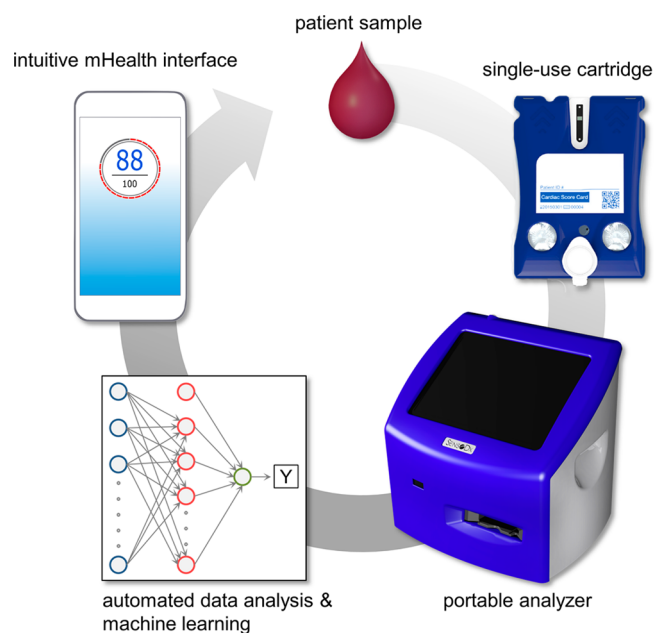


Figure 1. Intended use cycle of the p-BNC system for routine cardiac wellness testing.

efficient manner. The process begins by introducing a small quantity ($\sim 100\ \mu\text{L}$, 2 drops) of patient sample (e.g., serum, plasma, or oral fluids) into a disposable cartridge. The cartridge is inserted into the portable analyzer, which automatically performs the multistep assay sequence. Image analysis routines “digitize biology” by converting the signal into biomarker concentrations. The 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. Providing patients with personalized wellness information has the potential to promote prevention and active management of cardiac health, and the combination of high-sensitivity POC diagnostics and machine learning has the potential to transform health care moving forward. While this Account exclusively highlights the p-BNC system, more comprehensive reviews of the LOC field as a whole have been published by other groups.^{2,5,7,18}

■ SINGLE-USE MICROFLUIDIC CARTRIDGES

Critical to the “sensors that learn” concept are the sensor ensembles themselves and the platform technology that enables protein, antibody, small molecule, and oligonucleotide biomarker measurements at the POC. To illustrate how the p-BNC technology functions, Figure 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 where quantitation of proteins and antibodies outperforms ELISA, achieving lower limits of detection, faster

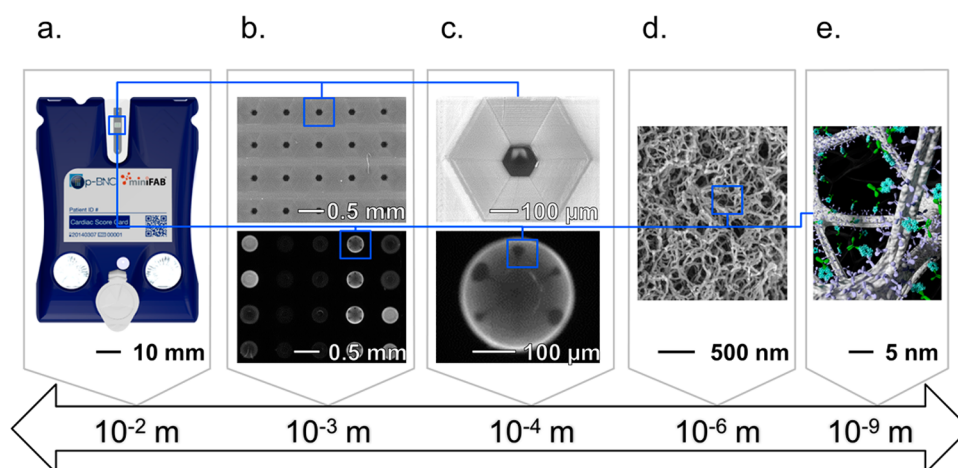


Figure 2. A graphical depiction of p-BNC features at various length scales: (a) The approximately credit-card sized p-BNC cartridge. (b) The p-BNC's 4×5 bead array with (bottom, fluorescence image) and without (top, SEM image) agarose bead sensors. (c) Magnified view of a single bead sensor (bottom, 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.

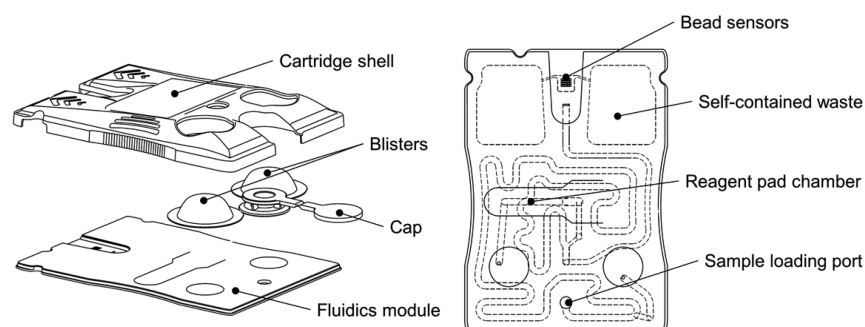


Figure 3. Illustrations of the p-BNC cartridge. The exploded diagram (left) reveals the main fluidics module onto which two reagent blister packs and an adhesive cap are mounted. The cartridge outer shell encloses and protects the cartridge. The fluid routing diagram (right) shows the location of the sample entry, user-configurable reagent pad chamber, self-containing waste chambers, and bead sensor array. Reproduced from ref 15 with permission from The Royal Society of Chemistry.

analysis times, and improved usability.¹³ The six-sided flow-through microcontainer design allows convective transport to penetrate the agarose beads, where short depletion layers improve capture efficiency relative to 2-D methods.¹⁹ The agarose beads' 3-D fibrous network that is indexed matched to water provides a distinct advantage over 2-D capture (e.g., lateral flow devices, planar microarrays, 96-well plates) because the 3-D lattice structure concentrates a higher density of immunocomplexes within the optical path of the fluorescence microscope, resulting in higher signal and, thus, improved sensitivity. Other advantages of this agarose bead system include (1) drastically reduced analysis times relative to diffusion-dominant ELISA, (2) the ability accommodate a wide range of target analyte properties (e.g., molecular weight, size, and shape) by customizing porosity, (3) a capacity to perform both two-site immunometric and competitive assay formats, and (4) low nonspecific binding characteristics provided by this polymerized sugar matrix. The first p-BNC designs were composed of anisotropically etched silicon 100 wafers. Recent efforts to translate these devices into clinical practice have resulted in the mass-produced, globally scalable, inexpensive injection molded plastic cartridges shown here.

A common obstacle for translating LOC devices from R&D laboratories to clinical practice is the absence of robust interfaces for the chip and its peripheral reagents. These

complicated “world-to-chip” interfaces render some LOC devices impractical outside of research settings, inheriting the title “chips in a lab”.^{6,7} The methods through which fluids (e.g., the specimen, bioreagents, aqueous buffers, and waste) are manipulated must be carefully considered when designing a microfluidic device. Further, successful LOC devices minimize manual fluid handling steps and perform preprocessing steps within the device.

The p-BNC microfluidic cartridge (Figure 3) streamlines various fluid handling steps. First, a small volume of biospecimen (i.e., serum, plasma, urine, or oral fluids) is introduced into the loading port. The sample fills the fluidic cartridge via capillary flow, and a passive valve governs automatic metering ($100 \mu\text{L}$) of sample volumes. After the sample is loaded, the user closes the device via an adhesive cap. “On-chip” processing of sample is completed by inline filters. The cartridge features two foil blister packs containing phosphate-buffered saline (PBS), which serve as the aqueous buffers. Puncturing mechanisms on the cartridge underneath the blisters rupture the foil upon external compression via the analyzer's automated blister actuation system. The right blister controls the antigen delivery pathway while the left blister controls the detecting antibody pathway. An easily accessible reagent pad chamber on the top face of the cartridge holds a glass fiber conjugate pad with detecting antibody reagents that

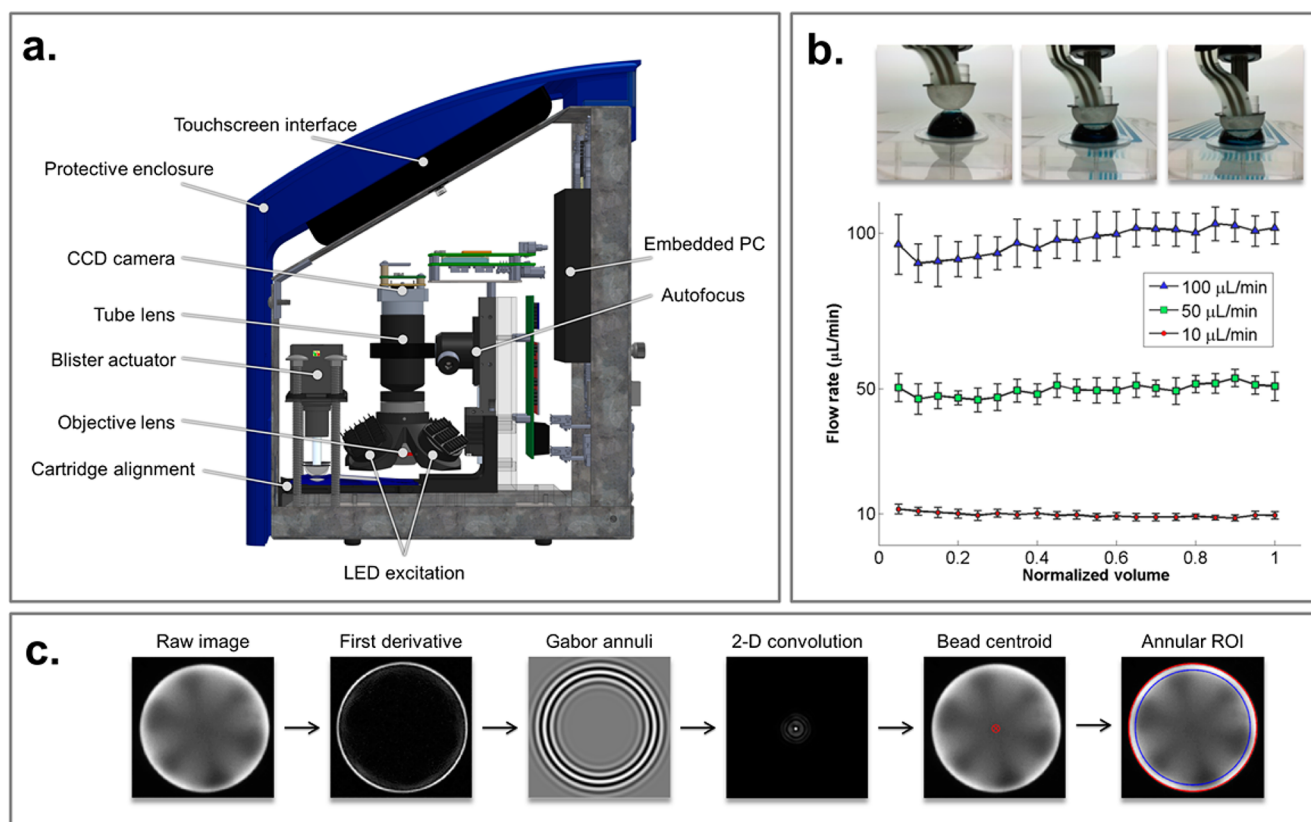


Figure 4. Portable and automated instrumentation for the p-BNC. (a) Illustration of the analyzer prototype (internal side view). (b) Automated fluid delivery system showing images of the actuator compressing a blister at various heights (top) and flow rate verification results (bottom) with mean flow rates and standard deviation (error bars) for five runs at each target flow rate. (c) Summary of the automated image analysis procedure, which locates, extracts, and averages the beads' fluorescence signal. Reproduced from ref 15 with permission from The Royal Society of Chemistry.

are eluted and delivered to the bead sensor array. Any unbound reagents and antigen are drained into waste chambers adjacent to the bead array. When the assay is complete, the cartridge may be removed from the instrument and safely disposed in a biohazardous waste container. The p-BNC's high level of fluidic integration reduces potential human errors, improves repeatability, and qualifies its use at the POC. Additionally, the automation of fluid handling steps allows p-BNC assays to be performed with minimal training.

■ PORTABLE AND AUTOMATED POINT-OF-CARE ANALYZER

Integrating microfluidic disposables and their associated instrumentation into a unified analysis system is a primary challenge for POC diagnostics.²⁰ In the case of fluorescence immunoassays, the optical detection system must be portable, inexpensive, and sensitive. Recent improvements in optoelectronics hardware like CMOS cameras and LEDs offer new low-cost and sensitive options for LOC instrumentation. Similarly, as the performance characteristics of single-board computers for embedded systems continue to improve, increasingly sophisticated software provides new opportunities for developers to improve device performance and user experience. The goal for developing a successful portable assay system is to create an automated workflow that requires no special training.

The p-BNC analyzer prototype described previously¹⁵ and shown in Figure 4 is approximately 22 cm × 22 cm × 30 cm ($l \times w \times h$), weighs less than 7 kg, and features a compact fluorescence imaging module, automated fluid delivery system,

cartridge alignment, embedded PC, and touchscreen interface (Figure 4a). The analyzer's miniaturized optical system is designed to image fluorescently labeled beads (AlexaFluor-488) and consists of an off-axis illumination module with four blue LEDs that are filtered (483 nm) and directed to the object plane with aspheric condenser lenses. The inline optical assembly comprises an objective lens (4×, 0.13 NA), an emission bandpass filter (535 nm), a tube lens, and a CCD imager attached to a precision positioning actuator for focusing. In addition to the compact fluorescence microscope, an automated fluid delivery system comprising two vertically oriented actuators compresses the cartridge's blister packs to perform customizable flow protocols. The cartridge slot contains a loading mechanism that aligns the cartridge with the blister actuators and optics field of view. The p-BNC software user interface is displayed and manipulated via an interactive touchscreen.

The method of motivating fluids within a microfluidic device is an important consideration when developing a new device concept. While there are a variety of driving forces to choose from, blister packs are a popular choice for POC settings because the aqueous buffers can be stored conveniently on the device. Several groups developing POC devices have selected blister pack actuation as their primary source of fluid motivation²¹ including commercial products such as the Abbott i-STAT device²² and the Daktari Diagnostics device.²³ Despite the numerous examples of blister pack actuation systems in the literature, little information regarding the accuracy and precision of the flow rates resulting from actuation have been

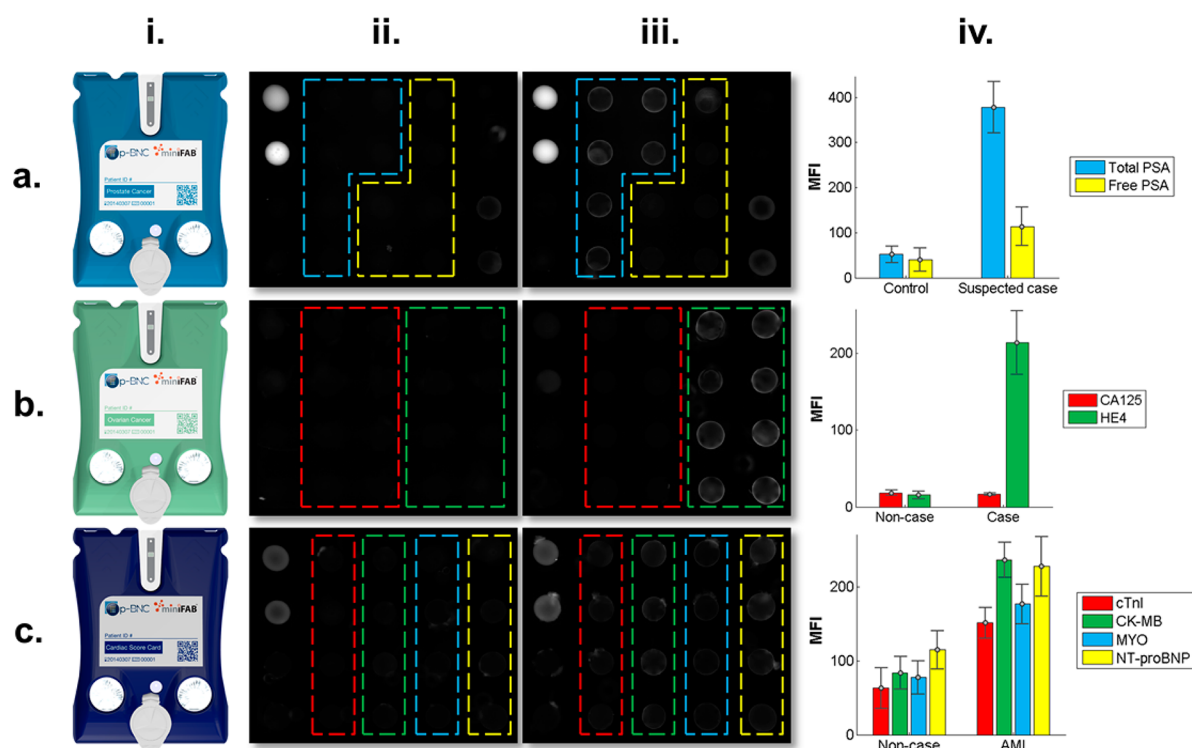


Figure 5. p-BNC's multifunctionality was demonstrated by completing assays within the single-use disposable cartridges (i). Fluorescence images are shown for noncases/controls (ii) and cases (iii) where each panel includes positive calibrators and negative controls at the left-most column of the bead array. Analytes were detected for each disease application; total PSA (blue) and free PSA (yellow) for prostate cancer (a); CA125 (red) and HE4 (green) for ovarian cancer (b); cTnI (red), CK-MB (green), MYO (blue), and NT-proBNP (yellow) for AMI diagnosis (c). Mean fluorescence intensities (iv) were determined from the annular ROI method with error bars showing the standard deviation from duplicate bead sensors from a single experiment. Reproduced from ref 15 with permission from The Royal Society of Chemistry.

published. Previously, our group showed that flow rate is an important parameter for optimizing analyte capture;¹⁹ therefore, delivering accurate and repeatable flow rates was a high priority when designing instrumentation.

Recently, we developed a method for delivering highly customizable and repeatable flow rates with blister actuation.¹⁵ Our fluid delivery system features two key innovations: blister burst detection and a dynamic blister actuation model. Force sensitive resistors are embedded underneath the blister actuator tips, and a burst detection algorithm recognizes the event when the blister has ruptured, allowing the automated fluid delivery system to track and administer accurate flow rates into the cartridge. Once the blister is burst, the actuators compress the blister with an actuation rate derived from a geometric model of the blister volume. The blister was mounted to an experimental microfluidic card with known volume, and video analysis software estimated the volumetric flow rate by measuring the velocity of a dye solution (Figure 4b). Flow rates at three target levels (100, 50, and 10 $\mu\text{L}/\text{min}$) were delivered for five runs each, demonstrating exceptional control of fluid delivery. The automation of sample and reagent fluids eliminates labor-intensive manipulations and improves repeatability and reproducibility en route to making quality standard measurements at the POC a reality.

Automated data analysis routines that would previously be considered too computationally intensive for portable POC systems are now feasible as single-board computers are becoming better, faster, and cheaper. In this manner, increasingly sophisticated software solutions are expected to relax, and perhaps displace altogether, certain design require-

ments resulting in LOC systems that are easier and cheaper to build. In addition to algorithmically controlling fluid delivery, the p-BNC analyzer automates the analysis of fluorescence micrographs, converting pixel intensities into biomarker concentrations.¹⁵ The image analysis software summarized in Figure 4c and applied to each bead individually uses novel computer vision methods to detect the bead's locations and analyze pixels within a region of interest (ROI). First, the finite difference image identifies the bead's outer edge. Next, beads within the expected size distribution are identified via generating several Gabor annuli²⁴ with slightly different properties and subsequently performing 2-D convolution operations on the derivative image. The resulting convolution responses are normalized and averaged, and the bead locations are defined by the maximum averaged response. The outer edge is determined by fitting a circle to the points of maximum intensity in the finite difference image. Next, an annular ROI is mapped along the bead's outer edge, the signal within the ROI is averaged, and a Grubbs' test removes outlier beads from the analysis. Lastly, the resulting mean fluorescent intensities (MFI) are converted to biomarker concentrations by referencing a predetermined dose curve (four- or five-parameter logistic curve).

To demonstrate the p-BNC system's bioassay flexibility, a proof of concept study was performed using the integrated and automated bioassay platform described previously.¹⁵ In development assays for the screenings of prostate and ovarian cancer and acute myocardial infarction (AMI) diagnosis were conducted on the p-BNC system using human serum samples for cases and controls (Figure 5). The prostate cancer screening

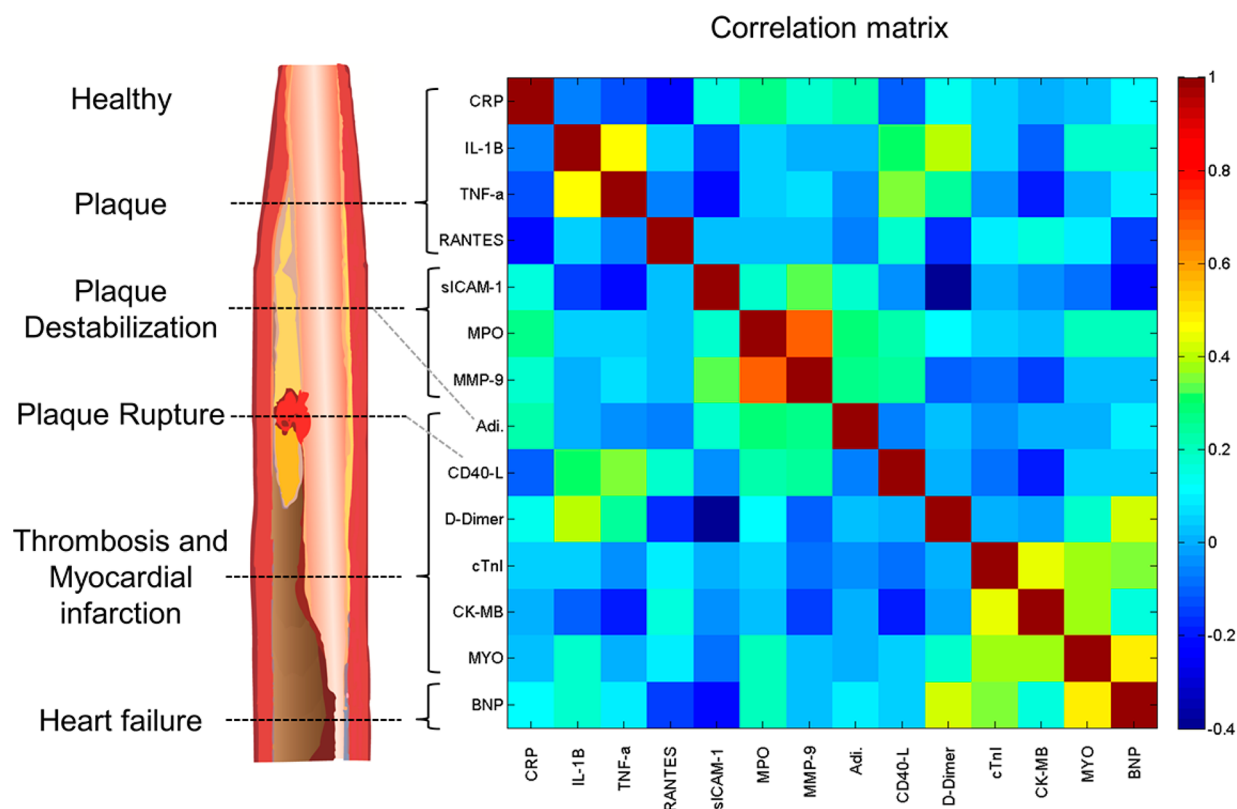


Figure 6. Cardiac biomarkers and their pathophysiological role in atherosclerotic plaque development. An idealized diagram (left) shows simplified stages of atherosclerotic plaque development and associated biomarkers. A correlation matrix (right) shows the Pearson correlation coefficient matrix for all patients ($N = 579$) and all patient outcomes in the study. Reproduced with permission from ref 16. Copyright 2016 Elsevier.

panel (Figure 5a) exhibits higher signal in both the total and free PSA beads for the suspected positive case, indicating that the p-BNC can distinguish between prostate cancer cases and controls. Similarly for ovarian cancer, the p-BNC (Figure 5b) distinguishes a late-stage cancer patient from a noncase patient via substantially higher signal in HE4 bead sensors. For AMI diagnosis (Figure 5c), all four cardiac biomarkers (cTnI, CK-MB, MYO, and NT-proBNP) are elevated in the AMI patient, suggesting the p-BNC's utility in distinguishing between AMI and noncase chest pain patients. This compilation of data showcases the p-BNC platform's flexibility in which a universal cartridge can be reconfigured via the spatial programming of bead sensors. While the work described here demonstrates the p-BNC's capacity for highly customizable multiplex panels to cover a number of disease indications, our prior work performed on less integrated instrumentation enumerates the assay performances for a selection of analyte targets¹⁷ and dedicated multiplex panels such as drugs of abuse,¹⁴ cardiac heart disease,²⁵ and ovarian cancer.¹²

■ CARDIAC SCORECARD FOR PREDICTING A SPECTRUM OF CARDIOVASCULAR DISEASES

The implementation of machine and statistical learning in diagnostic devices has the potential to radically alter the way we quantify our health, and once adopted clinically these devices could outperform current standard methods for diagnosing and determining prognosis of diseases.²⁶ Not only will the composite information on multiplex biomarker panels outperform any single-marker diagnostic test, but also the introduction of new disease scores and classifications provides a result that is more interpretable than simply a list of

biomarker concentration values. The bridge between integrated POC testing and model development is critical for translating theoretical approaches into mainstream clinical practice. In this capacity, our group is developing the Cardiac ScoreCard, statistical learning models that predict cardiovascular diseases using data from multiplex cardiac marker panels and risk factor information.¹⁶ CVD is the leading cause of death in the U.S. and globally, and the exorbitant healthcare cost attributed to CVD is burdening the U.S. economy. The joint goal of saving lives and reducing healthcare costs may be accomplished by shifting the focus from late-stage disease maintenance to early detection and prevention of CVD.

Clinical decision support systems (CDSSs) are support tools that assist in medical decisions by providing clinicians with personalized assessments or recommendations²⁷ and offer a promising solution for managing CVD. Several CDSSs for CVD have been developed, featuring various machine-learning methods such as artificial neural networks,²⁸ Support Vector Machines,²⁹ random forest,³⁰ Bayesian networks,³¹ logistic regression,³² and ensemble methods.³³ Although CDSSs promise enhanced diagnostic results, shorter wait times, and reduced cost versus the standard of care, physicians may be hesitant to implement "black box" CDSSs (i.e., the algorithm's results and methods to obtain them are either uninterpretable or not capable of providing actionable therapeutic recommendations). Therefore, the Cardiac ScoreCard uses a lasso logistic regression approach, converting risk factors and biomarker data into a single score with interpretable and clinically useful information in the form of logistic regression coefficients. When fully developed, the Cardiac ScoreCard is intended to provide personalized cardiac health assessments

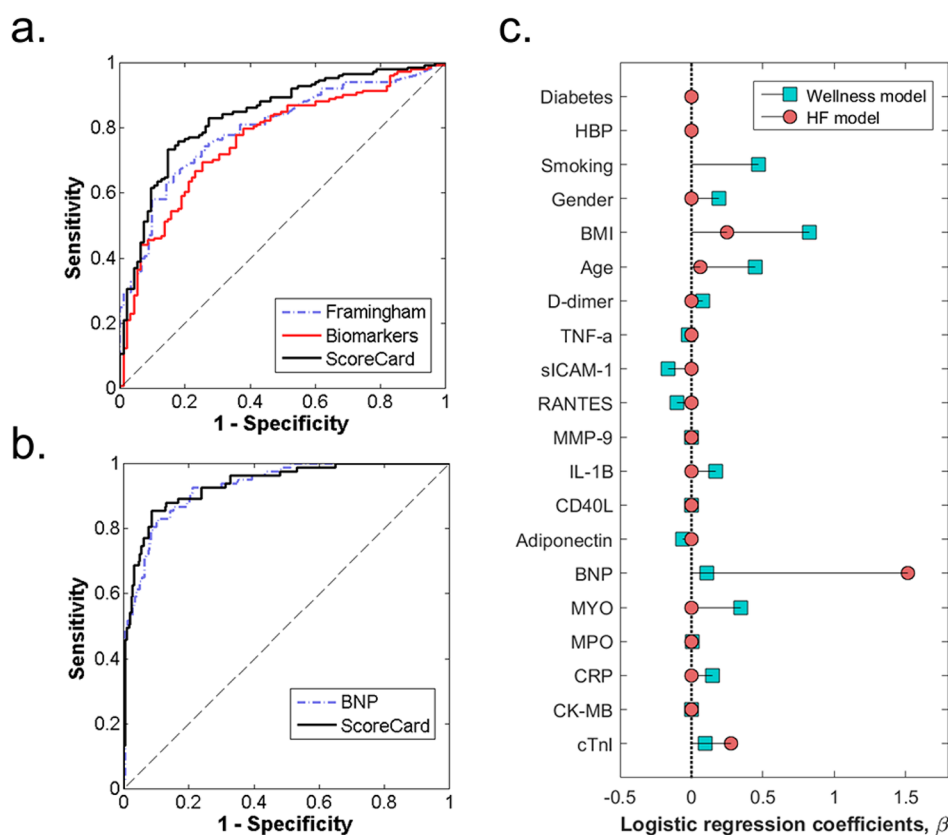


Figure 7. Results of the Cardiac ScoreCard and various reference methods. (a) Receiver operating characteristic (ROC) curves for the cardiac wellness application (high versus low risk) for the Framingham 10-year risk score, a biomarkers-only model, and the Cardiac ScoreCard. (b) ROC curves for HF diagnosis using a single-marker BNP test and the Cardiac ScoreCard. (c) Lasso logistic regression coefficients for the Cardiac ScoreCard wellness and HF models. Reproduced with permission from ref 16. Copyright 2016 Elsevier.

across a spectrum of CVD with predictive models for cardiac wellness, AMI diagnosis, AMI prognosis, heart failure (HF) diagnosis, and HF prognosis. In this Account, we report our recent results for cardiac wellness and HF diagnosis,¹⁶ while the others will be featured in future publications.

The Cardiac ScoreCard assay comprises a multiplex panel of biomarkers from a diverse pathophysiology for two reasons (Figure 6). First, others have demonstrated that a multimarker strategy with pathobiologically diverse biomarkers improves performance in CVD risk prediction.³⁴ Further, a selection of markers that are differentially expressed across various phases of atherosclerotic plaque development provides new opportunities for CVD prediction models. Second, it is important to train the models with predictors that are uncorrelated. When biomarkers from the same pathophysiology are selected for a model, the predictors tend to be highly correlated. Conversely, selecting biomarkers from a diverse background are expected to increase the overall information content in the predictive model.

A model for assessing and predicting overall heart health for consumer wellness testing was recently developed and described previously.¹⁶ Briefly, the wellness ScoreCard model (AUC = 0.84) outperformed both the Framingham 10-year CVD risk score (AUC = 0.80) and a biomarker-only model (AUC = 0.77) in terms of discrimination between high risk and low risk patient groups (Figure 7a). Additionally, the ScoreCard model showed good calibration across deciles of predicted risk (Hosmer–Lemeshow p = 0.98), demonstrating its usefulness as a score for wellness. Similarly, a HF diagnosis model was developed and compared with a standard method for diagnosis

(BNP test). The multivariate ScoreCard algorithm showed better discrimination in diagnosing HF than the single-marker BNP score (AUC = 0.94 and 0.93, respectively) (Figure 7b), suggesting that the diagnosis of HF can be enhanced by adding auxiliary biomarkers and patient demographics.

One advantageous feature of lasso logistic regression is automatic feature selection.³⁵ Feature selection reduces model complexity and improves generalization by discarding unnecessary predictors while retaining the most relevant, and the lasso logistic regression method performs this feature selection by shrinking the regression coefficients. Not only does this feature selection approach improve prediction performance, but it also provides assay developers with a prioritized list of biomarker candidates for future implementation in multimarker panels. Figure 7c shows the logistic regression coefficients for both the wellness and HF models. In the wellness model, the lasso method selected 15 predictors (i.e., nonzero coefficients) with BMI ($\beta_{\text{BMI}} = 0.82$), smoking ($\beta_{\text{smoking}} = 0.47$), age ($\beta_{\text{age}} = 0.45$), myoglobin ($\beta_{\text{MYO}} = 0.34$), gender ($\beta_{\text{gender}} = 0.19$), and IL-1 β ($\beta_{\text{IL-1}\beta} = 0.17$) having the largest effect sizes. These results suggest that the discrimination between high and low risk patients is contingent on a relatively large number of predictors, comprising demographics and biomarkers from a diverse pathophysiology. On the other hand, the lasso method returned a sparse model for HF diagnosis with only four nonzero predictors: BNP ($\beta_{\text{BNP}} = 1.51$), cTnI ($\beta_{\text{cTnI}} = 0.28$), BMI ($\beta_{\text{BMI}} = 0.25$), and age ($\beta_{\text{age}} = 0.06$). From a practical assay development perspective, this sparse HF model, made possible through lasso-based feature selection methods, has the potential

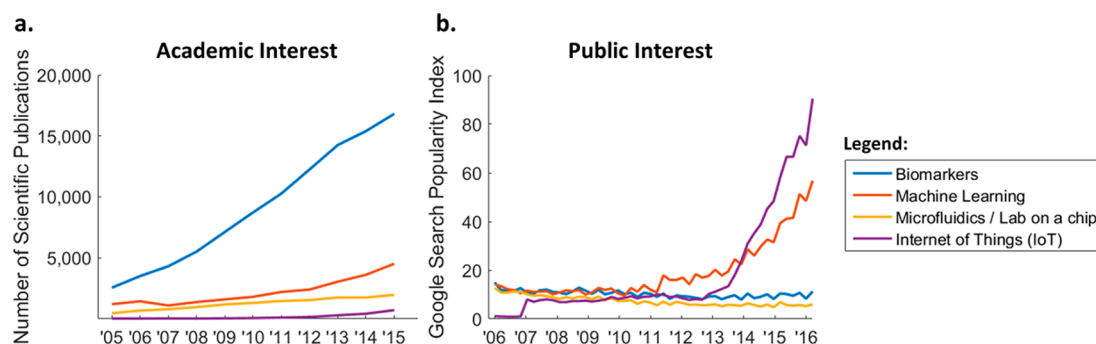


Figure 8. Academic and public interests in various topics over the past decade: (a) Number of scientific publications reported by the Web of Science (<http://www.webofscience.com>). (b) Relative popularity of topics in the United States (total searches for the topic out of all Google searches) reported by Google Trends (<http://www.google.com/trends>).

to significantly reduce the cost of reagents and simplify the assay chemistry.

The importance of translating biomarker data into interpretable wellness scores is further underscored by the disparity between academic and general public interests. In the past decade, over 100 000 biomarker papers have been published with the number of biomarker papers increasing every year; however, the relative public interest in biomarkers appears to be declining over time (Figure 8). To bridge the gap, researchers must convey biomarker information more effectively to the general population by developing intuitive health report cards, like the Cardiac ScoreCard. The Cardiac ScoreCard technology will provide a comprehensive biomarker panel capable of assessing early risk as well as monitoring late stage disease progression for AMI and HF patients. These marker-driven tests have the potential to radically reduce costs, decrease wait times, and add new options for patients needing regular health monitoring. 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 biomarkers and IoT infrastructures with predictive analytics may exponentially improve drug discovery and health policy and allow new options for personalized wellness management.

CONCLUSIONS AND OUTLOOK

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. However, while the application of AI in medicine is still in its infancy, several challenges remain for these integrated chem- and biosensing strategies to reach their full potential. 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. Integrated chem- and biosensor platform developers need to design universal systems that are compatible with the 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., artificial neural networks and SVMs) and

provide uninterpretable results. 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. The combination of a platform to digitize biology and predictive analytics can change 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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Notes

The authors declare the following competing financial interest(s): Principal Investigator, John T. McDevitt, has an equity interest in SensoDX, LLC., and also serves on the Scientific Advisory Board.

Biographies

Michael P. McRae received his B.S. in Biomedical Engineering from Saint Louis University. He is currently a Ph.D. candidate in Bioengineering at Rice University under the guidance of John T. McDevitt. His research focuses on developing integrated and automated instrumentation for microfluidic devices and the applications of machine learning in novel biosensor systems.

Glenn Simmons has 15 years of experience developing medical microdevices that function to improve the speed, accuracy, and affordability of disease diagnosis. Mr. Simmons is the project lead for the design and development of the p-BNC cartridge and assay development on the system. Mr. Simmons holds a B.S. in Chemistry from the University of Texas at Austin.

Dr. Jorge Wong earned his Ph.D. in Chemistry at The University of Texas at Austin. His research is focused on the development of bioassays on the p-BNC sensor platform.

Dr. John T. McDevitt is a pioneer in the development of the p-BNC sensor systems. Further, over the past 5 years, the McDevitt laboratory has secured in-depth experience with management and execution of international, multisite clinical trials that have resulted in the collection of unique databases using these p-BNC tools. Dr. McDevitt currently serves as the Chair for the Biomaterials Department at New York University's College of Dentistry and has served as the scientific founder of multiple diagnostic companies.

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■ REFERENCES

- (1) Forsman, R. W. Why is the laboratory an afterthought for managed care organizations? *Clin. Chem.* **1996**, *42*, 813–816.
- (2) Whitesides, G. M. The origins and the future of microfluidics. *Nature* **2006**, *442*, 368–373.
- (3) Becker, H. One size fits all? *Lab Chip* **2010**, *10*, 1894–1897.
- (4) Manz, A.; Graber, N.; Widmer, H. M. Miniaturized total chemical analysis systems: a novel concept for chemical sensing. *Sens. Actuators, B* **1990**, *1*, 244–248.
- (5) Yager, P.; Edwards, T.; Fu, E.; Helton, K.; Nelson, K.; Tam, M. R.; Weigl, B. H. Microfluidic diagnostic technologies for global public health. *Nature* **2006**, *442*, 412–418.
- (6) Mohammed, M. I.; Haswell, S.; Gibson, I. Lab-on-a-chip or chip-in-a-lab: challenges of commercialization lost in translation. *Procedia Technology* **2015**, *20*, 54–59.
- (7) Chin, C. D.; Linder, V.; Sia, S. K. Commercialization of microfluidic point-of-care diagnostic devices. *Lab Chip* **2012**, *12*, 2118–2134.
- (8) Becker, H. Lost in translation. *Lab Chip* **2010**, *10*, 813–815.
- (9) Jordan, M. I.; Mitchell, T. M. Machine learning: trends, perspectives, and prospects. *Science* **2015**, *349*, 255–260.
- (10) Patel, V. L.; Shortliffe, E. H.; Stefanelli, M.; Szolovits, P.; Berthold, M. R.; Bellazzi, R.; Abu-Hanna, A. The coming of age of artificial intelligence in medicine. *Artif. Intell. Med.* **2009**, *46*, 5–17.
- (11) Jokerst, J. V.; Jacobson, J. W.; Bhagwandin, B. D.; Floriano, P. N.; Christodoulides, N.; McDevitt, J. T. Programmable nano-bio-chip sensors: analytical meets clinical. *Anal. Chem.* **2010**, *82*, 1571–1579.
- (12) Shadfan, B. H.; Simmons, A. R.; Simmons, G. W.; Ho, A.; Wong, J.; Lu, K. H.; Bast, R. C., Jr.; McDevitt, J. T. A multiplexable, microfluidic platform for the rapid quantitation of a biomarker panel for early ovarian cancer detection at the point-of-care. *Cancer Prev. Res.* **2015**, *8*, 37–48.
- (13) Jokerst, J. V.; Chou, J.; Camp, J. P.; Wong, J.; Lennart, A.; Pollard, A. A.; Floriano, P. N.; Christodoulides, N.; Simmons, G. W.; Zhou, Y.; Ali, M. F.; McDevitt, J. T. Location of biomarkers and reagents within agarose beads of a programmable bio-nano-chip. *Small* **2011**, *7*, 613–624.
- (14) Christodoulides, N.; De La Garza, R., II; Simmons, G. W.; McRae, M. P.; Wong, J.; Newton, T. F.; Smith, R.; Mahoney III, J. J.; Hohenstein, J.; Gomez, S.; Floriano, P. N.; Talavera, H.; Sloan, D. J.; Moody, D. E.; Andrenyak, D. M.; Kosten, T. R.; Haque, A.; McDevitt, J. T. Application of programmable bio-nano-chip system for the quantitative detection of drugs of abuse in oral fluids. *Drug Alcohol Depend.* **2015**, *153*, 306–313.
- (15) McRae, M. P.; Simmons, G. W.; Wong, J.; Shadfan, B.; Gopalkrishnan, S.; Christodoulides, N.; McDevitt, J. T. Programmable bio-nano-chip system: a flexible point-of-care platform for bioscience and clinical measurements. *Lab Chip* **2015**, *15*, 4020–4031.
- (16) McRae, M. P.; Bozkurt, B.; Ballantyne, C. M.; Sanchez, X.; Christodoulides, N.; Simmons, G.; Nambi, V.; Misra, A.; Miller, C. S.; Ebersole, J. L.; Campbell, C.; McDevitt, J. T. Cardiac ScoreCard: a diagnostic multivariate index assay system for predicting a spectrum of cardiovascular disease. *Expert Syst. Appl.* **2016**, *54*, 136–147.
- (17) Chou, J.; Wong, J.; Christodoulides, N.; Floriano, P.; Sanchez, X.; McDevitt, J. T. Porous bead-based diagnostic platforms: bridging the gaps in healthcare. *Sensors* **2012**, *12*, 15467–15499.
- (18) Yetisen, A. K.; Akram, M. S.; Lowe, C. R. Paper-based microfluidic point-of-care diagnostic devices. *Lab Chip* **2013**, *13*, 2210–2251. Dittrich, P. S.; Tachikawa, K.; Manz, A. Micro total analysis systems: latest advancements and trends. *Anal. Chem.* **2006**, *78*, 3887–3908.
- (19) Chou, J.; Li, L. E.; Kulla, E.; Christodoulides, N.; Floriano, P. N.; McDevitt, J. T. Effects of sample delivery on analyte capture in porous bead sensors. *Lab Chip* **2012**, *12*, S249–S256.
- (20) Wootton, R. C. R.; deMello, A. J. Microfluidics: Exploiting elephants in the room. *Nature* **2010**, *464*, 839–840. West, J.; Becker, M.; Tombrink, S.; Manz, A. Micro total analysis systems: latest achievements. *Anal. Chem.* **2008**, *80*, 4403–4419.
- (21) Qiu, X.; Thompson, J. A.; Chen, Z.; Liu, C.; Chen, D.; Ramprasad, S.; Mauk, M. G.; Ongagna, S.; Barber, C.; Abrams, W. R.; Malamud, D.; Corstjens, P. L. A. M.; Bau, H. H. Finger-actuated, self-contained immunoassay cassettes. *Biomed. Microdevices* **2009**, *11*, 1175–1186. Selvakumar, S.; Linares, R.; Oppenheimer, A.; Anthony, B. Variation analysis of flow rate delivered using a blister pump. *Proc. SPIE* **2012**, *8251*, 82510I.
- (22) Abbott Point of Care, Inc. <http://www.abbottpointofcare.com/Patient-Care-Settings/Hospital.aspx> (accessed March 1, 2016).
- (23) Daktri Diagnostics <http://daktridx.com/products/> (accessed March 1, 2016).
- (24) Rhodes, A.; Bai, L. Circle detection using a gabor annulus. *Proc. Br. Mach. Vis. Conf.* **2011**, 108.1.
- (25) Christodoulides, N.; Floriano, P. N.; Sanchez, X.; Li, L.; Hocquard, K.; Patton, A.; Muldoon, R.; Miller, C. S.; Ebersole, J. L.; Redding, S.; Yeh, C. K.; Furmaga, W. B.; Wampler, D. A.; Bozkurt, B.; Ballantyne, C. M.; McDevitt, J. T. Programmable bio-nanochip technology for the diagnosis of cardiovascular disease at the point-of-care. *Methodist DeBakey Cardiovasc. J.* **2012**, *8*, 6–12.
- (26) Zhang, Z. An in vitro diagnostic multivariate index assay (IVDMIA) for ovarian cancer: harvesting the power of multiple biomarkers. *Rev. Obstet. Gynecol.* **2012**, *5*, 35–41.
- (27) Kawamoto, K.; Houlihan, C. A.; Balas, E. A.; Lobach, D. F. Improving clinical practice using clinical decision support systems: a systematic review of trials to identify features critical to success. *Br. Med. J.* **2005**, *330*, 765.
- (28) Baxt, W. G. Use of an artificial neural network for the diagnosis of myocardial infarction. *Ann. Intern. Med.* **1991**, *115*, 843–848. Yan, H.; Jiang, Y.; Zheng, J.; Peng, C.; Li, Q. A multilayer perceptron-based medical decision support system for heart disease diagnosis. *Expert Syst. Appl.* **2006**, *30*, 272–281.
- (29) Shilaskar, S.; Ghatol, A. Feature selection for medical diagnosis: evaluation for cardiovascular diseases. *Expert Syst. Appl.* **2013**, *40*, 4146–4153.
- (30) Ion Titapiccolo, J.; Ferrario, M.; Cerutti, S.; Barbieri, C.; Mari, F.; Gatti, E.; Signorini, M. G. Artificial intelligence models to stratify cardiovascular risk in incident hemodialysis patients. *Expert Syst. Appl.* **2013**, *40*, 4679–4686.
- (31) Vila-Francés, J.; Sanchis, J.; Soria-Olivas, E.; Serrano, A. J.; Martínez-Sober, M.; Bonanad, C.; Ventura, S. Expert system for predicting unstable angina based on Bayesian networks. *Expert Syst. Appl.* **2013**, *40*, 5004–5010.
- (32) Blankenberg, S.; Zeller, T.; Saarela, O.; Havulinna, A. S.; Kee, F.; Tunstall-Pedoe, H.; Kuulasmaa, K.; Yarnell, J.; Schnabel, R. B.; Wild, P. S.; Münzel, T. F.; Lackner, K. J.; Tiret, L.; Evans, A.; Salomaa, V. Contribution of 30 biomarkers to 10-year cardiovascular risk estimation in 2 population cohorts: the MONICA, Risk, Genetics, Archiving, and Monograph (MORGAM) Biomarker Project. *Circulation* **2010**, *121*, 2388–2397.
- (33) Das, R.; Turkoglu, I.; Sengur, A. Effective diagnosis of heart disease through neural networks ensembles. *Expert Syst. Appl.* **2009**, *36*, 7675–7680.
- (34) Morrow, D. A.; Braunwald, E. Future of biomarkers in acute coronary syndromes: moving toward a multimarker strategy. *Circulation* **2003**, *108*, 250–252. Sabatine, M. S.; Morrow, D. A.; de Lemos, J. A.; Gibson, C. M.; Murphy, S. A.; Rifai, N.; McCabe, C.; Antman, E. M.; Cannon, C. P.; Braunwald, E. Multimarker approach to risk stratification in non-ST elevation acute coronary syndromes: simultaneous assessment of troponin I, C-reactive protein, and B-type natriuretic peptide. *Circulation* **2002**, *105*, 1760–1763.

(35) Hastie, T.; Tibshirani, R.; Friedman, J. H. *The Elements of Statistical Learning: Data Mining, Inference, and Prediction*; Springer Verlag: Berlin, 2009; pp 68–73.