Programmable bio-nano-chip system: a flexible point-of-care platform for bioscience and clinical measurements

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Abstract

The development of integrated instrumentation for universal bioassay systems serves as a key goal for the lab-on-a-chip community as a whole. Likewise, the programmable bio-nano-chip (p-BNC) system is a versatile multiplexed and multiclass chem- and bio-sensing system 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 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 indexed 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 point-of-care. A companion portable analyzer prototype has also been developed so as to integrate fluid motivation, optical detection, and automated data analysis, and serve as the human interface for complete assay automation. In this report, we provide a systems-level perspective of the p-BNC universal biosensing platform with an emphasis on fluid motivation, device integration, and automation. To demonstrate the flexibility of the p-BNC, we distinguish disease and non-case patients across three significant disease applications: prostate cancer, ovarian cancer, and acute myocardial infarction. A rapid (~7 minute) myoglobin assay was developed using the fully automated p-BNC system.
Introduction

Significant progress has been made towards the development of lab-on-a-chip (LOC) devices over the years serving a variety of applications. However, few of these devices have obtained widespread commercial and clinical adoption. Traditionally, microfluidics research has relied on cumbersome equipment like syringe pumps and microscopes which are not amenable to point-of-care (POC) settings. Integration of microfluidic and instrumentation components into unified system remains a central challenge for POC diagnostics. 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) 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. Similarly challenging is creating an automated workflow compatible for non-experts with limited to no human intervention required between sample introduction and data presentation.

A primary design consideration for LOC systems 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, acoustic) for many purposes (to mix, react, detect, analyze, separate, etc.). In particular, 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. In this configuration, fluid flow may be initiated by finger actuation or controlled by an analyzer providing blister actuation. Aside from blood glucose measurements perhaps the most successful POC diagnostic 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. Similarly, Daktari Diagnostics is using a blister actuation approach for their CD4+ and CD8+ T lymphocyte counting device. Similarly, our group also has prior experience using blisters for on-chip storage and motivation of fluids. 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. Little information is currently 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.

Outside of integrated blister-pack LOC structures, several groups have demonstrated significant progress towards integration and automation of assay systems for use in remote settings. For example, Sia and colleagues have developed a low-cost mobile device with laboratory-quality results for testing HIV in remote settings. Cho and coworkers created a fully integrated lab-on-a-disc and portable analyzer that performs simultaneous biochemical analyses and immunoassays from whole blood. The Walt group has designed a platform for automated and multiplexed testing of salivary samples for the diagnosis of respiratory diseases. The Wheeler group developed an immunoanalyzer based on a digital microfluidics platform. Bau and colleagues designed a fully automated reader and a self-contained disposable cassette for nucleic acid detection. Singh and coworkers developed a microchip electrophoretic immunoassay and associated instrumentation for the quantitation of protein biomarkers in saliva. 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. Ligler et al. developed an easy-to-
use portable array biosensor system that can analyze multiple samples for multiple analytes. 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 based fluorescence microscope with machine learning software for automated detection of Giardia cysts. Yager’s work integrates low cost paper-based devices and cell phone imaging for the goal of increasing access and affordability to healthcare. The Whitesides group has developed a handheld device that performs electrochemical testing and transmits data to “the cloud” using a mobile phone. Demirci and coworkers are using cell phone imaging with microchip ELISA to detect the ovarian cancer marker HE4 at the POC.

While there are numerous successful applications of LOC devices targeting single application verticals, there remains less progress in creation of broadly responsive universal bioassay systems for POC use. The expense and time associated with the design, fabrication, testing, and validation serve as barriers for dedicated single class bioassay systems. Clearly, 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 report, we provide a systems-level perspective of the programmable bio-nano-chip (p-BNC) technology with an emphasis on fluid motivation, device integration, and automation. The cartridge design optimization and rationale will be published elsewhere. The versatility of the device is highlighted by the detection of 8 different analytes isolated from human serum samples across three disease applications, prostate cancer, ovarian cancer, and acute myocardial infarction (AMI). Further, the device’s analytical characteristics are demonstrated for a rapid myoglobin assay.

Materials and methods

Materials

The p-BNC cartridge design was inspired by laminate-based and fully integrated prototypes described previously. The injection molded plastic disposable cartridges for this study were designed and manufactured by MiniFAB (Scoresby, Victoria, Australia). 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), 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). High-power blue (490 nm) LEDs (part no. M490D2), aspheric condenser lenses (f = 20 mm) (part no. ACL2520-DG15-A), and SM1 lens tubes (part no. SM1L05) were purchased from Thorlabs (Newton, NJ, U.S.A.). LED heat sinks (part no. LPD25-4B), mounting tape (part no. LT-03), and 350 mA BuckPuck™ DC LED drivers (part no. 3021-D-I-350) were purchased from Luxeon Star LEDs (Brantford, Ontario, Canada). Size 14 captive linear actuators (part no. 35H4N-2.33-907) were purchased from Haydon Kerk Motion Solutions, Inc. (Waterbury, CT, U.S.A.). EasyDriver stepper motor drivers (part no. ROB-10267), an Arduino Pro Mini microcontroller board (part no. DEV-11113), FTDI cable (part no. DEV-09718), and 0.5” force sensitive resistors (part no. SEN-09375) were purchased from
SparkFun Electronics (Niwot, CO, U.S.A.). LM358 op-amps (part no. LM358NFS-ND) were purchased from Digi-Key (Thief River Falls, MN, U.S.A.). 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 custom printed circuit board (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 cover 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.

**Verification of flow rates**

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 thermforming 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 double-sided adhesive (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 (Supplemental Fig. S1). Top and bottom capping layers were made from 3M™ AF4300 polyethylene terephthalate (PET) (3M Company, St. Paul, MN, U.S.A.), 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 (Supplemental Fig. S2). Five runs at each target flow rate (10, 50, and 100 µL/min) were performed. Blisters were filled with 1 mL of dye, 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.

**Experimental**

*Device multi-functionality*
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, 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 antibodies were conjugated to the bead sensors by reductive amination for target capture, and matched pairs were conjugated to AlexaFluor-488® following manufacturer protocols. Details of the prostate cancer testing will be reported elsewhere. For ovarian cancer, serum samples were obtained from a recent study, and a 2-plex panel was developed using protocols previously described. The panel, containing cancer antigen 125 (CA-125) and human epididymis protein 4 (HE4) bead sensors, attempts to distinguish between a healthy female control and a late-stage ovarian cancer patient. Samples for cardiac testing were obtained from a recent clinical study following chest pain patients who presented to the Emergency Department (ED) with symptoms of acute coronary syndrome. 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. Details of the cardiac assay will be reported elsewhere. All fluid manipulations were handled by the portable analyzer. 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 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 the annular ROI method where the signal was averaged within the annulus between the bead outer diameter and 90% of the bead radius from the center.

**Rapid myoglobin assay**

MYO-specific antibodies and standards were acquired from Meridian Life Sciences Inc. (Memphis, TN). The biomarker C-reactive protein (CRP) was included in the panel as a control, and specific antibodies and standards were acquired from Fitzgerald Industries International (Acton, Massachusetts). AlexaFluor-488® was conjugated to MYO (clone 4E2) and CRP antibodies using AlexaFluor-488® protein labeling kit (Invitrogen, Eugene, Oregon). Goat anti Mouse IgG (H + L) (R-PE) antibodies from Fitzgerald Industries International (Acton, Massachusetts) were conjugated to the beads as positive controls, and the same antibody conjugated to AlexaFluor-488® was used as focal aids in the device. Reagent pads were functionalized with a cocktail of detecting antibody reagents and installed in the cartridge’s reagent pad chamber. Four of each bead type (MYO, CRP, positive calibrator, negative calibrator, and focal aid) 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 and gain of 1. Median pixel value of the background, defined as the entire image excluding the bead sensors, was subtracted for all images. MFI was calculated for each biomarker type via the annular ROI method where the signal was averaged within the annulus between the bead outer diameter and 50% of the bead radius from the center. A dose response curve (5-parameter logistic) was constructed for MYO using a concentration range 3.2 ng/mL to 50 μg/mL. Intra- and inter-assay
precision was assessed via replicate (N = 5) measurements of Liquichek™ Cardiac Markers Plus Control (Bio-Rad Laboratories, Inc., Hercules, CA).

**Results and discussion**

**p-BNC system**

Fig. 1 shows the p-BNC assay system comprised of a disposable assay cartridge and portable analyzer. The disposable cartridge (Fig. 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 ease of use at the POC. The bio-specimen (e.g., serum or saliva) is introduced into the sample loading port on the cartridge. A conjugate pad with dried reagents (e.g., detecting antibody) may be embedded in the cartridge and dissolved as needed through the activation of buffer-containing blister-packs. The bead-based labcard device contains analyte-specific antibodies bound to agarose beads supported in a plastic microchips. This mini-sensor ensemble is capable of multiplexing fluorescence immunoassays for proteins, oligonucleotides, and small molecules, outperforming laboratory-based enzyme-linked immnosorbent assay (ELISA) in terms of analysis time, limits of detection, and ease-of-use. 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 (Fig. 1B) optically images the p-BNC’s bead sensors and automates sample handling that would otherwise have been performed by a trained lab technician. Further, the analyzer performs automated data analysis on the acquired images, converts the image into usable data (i.e., biomarker concentration), and relays the data to disease-specific machine learning algorithms for diagnosis and prognosis. 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.

**Fig. 1.** The p-BNC system showing disposable cartridge (A) with bead sensors (magnified) and portable analyzer (B).
Disposable 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. 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. Fig. 2 depicts the cartridge’s major features and internal microfluidic circuitry. The device is constructed as an injection molded cartridge body capped with adhesive layers and sealed in a protective shell. A sample loop, passive valve bubble traps, debris filters, and mixers are incorporated in line for sample and reagent processing. Two foil blister packs containing buffers serve as the on-chip fluid source required to complete the various assays. Blister puncturing mechanisms on the molded layer rupture the blisters upon actuation and facilitate fluid flow into the cartridge. The user-configurable reagent pad chamber is accessed easily through an opening on the top face of the card, allowing the assay to be reconfigured with the reagents needed to complete the bioassay. The sensor region shown here is comprised of a 4 × 5 array of microwells which are “programmed” by the placement of analyte-specific agarose bead sensors. 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. A detailed description of the cartridge design will be covered in a future report.

Portable analyzer

The p-BNC analyzer is approximately 9” × 9” × 12” (length × width × height), weighs less than 15 lbs (6.8 kg), and has four main functions: fluid motivation, optical detection, automated data analysis, and human interface. Fig. 3 highlights the major components that facilitate these functions.
Fig. 2. The p-BNC disposable cartridge exploded diagram (left) and assembled top view (right).

Fig. 3. Perspective view of the p-BNC analyzer showing major features.
Fluid motivation

The p-BNC system 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/or evaporation. Upon actuation, the blister packs rupture and release their contents into the cartridge in a controlled fashion. Two key innovations have ensured repeatability of these events: force sensitive feedback for detecting the blister burst and geometry-adjusted actuation rate for delivering accurate and constant flow rates. The blister actuation module features two linear actuators oriented vertically over each of the cartridge’s blisters. A 3-D printed plastic mount supports and aligns the actuators over the cartridge. Force sensitive resistors (FSRs) detect when the actuator tip makes contact with the blisters and when the blister bursts, releasing its contents into the cartridge. Dome-shaped blister actuator tips attached to the surface of the FSRs facilitate the actuator’s mechanical interface with the blisters.

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. 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 we developed a mathematical model of blister and actuator geometry interaction 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.

Here, blister actuation was modeled via the interaction of two spherical caps (Fig. 4A) corresponding to the blister and the actuator tip. The total volume of the blister is given by the volume of the spherical cap in Eq. 1.

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

Eq. 1

where \( r \) 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 (Fig. 4B). 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, we simplify the problem by assuming the blister and actuator 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 \( z/2 \). Then, the volume ejected per step of the linear actuator is

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

Eq. 2

The volumetric flow rate is given by \( Q = \frac{dV}{dz} \times \frac{dz}{dt} \), where the desired constant flow rate \( Q \) is generated by applying the actuation rate, \( \frac{dz}{dt} \). 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 finally adjusted using a scaling factor to achieve constant flow rate at the desired target.

To verify the actuation method, flow rates were measured by tracking the fluid velocity of dye ejected from prototype blisters using a custom designed microfluidic card with meandering channels (Supplemental Fig. S1). Fig. 4C shows the results obtained using custom video analysis software. High, medium, and low flow rates (100, 50, and 10 μL/min) were targeted by the blister actuator software because these flow rates represent those typically used in an assay. The average flow rates measured were 97.72, 49.73, and 9.62 μL/min with CV 3.80%, 4.18%, and 7.64% for 100, 50, and 10 μL/min, respectively. Relatively low CVs and constant flow rate profiles in Fig. 4C demonstrate adequate control over fluid delivery using this blister actuation approach.

![Fig. 4. Spherical cap geometry (A), illustration of blister actuation model (B), and flow rate verification (C). Plotted are mean flow rates and standard deviation (error bars) for five runs at each target flow rate.](image)

**Optical detection**
The p-BNC analyzer uses a miniature fluorescence microscope system for imaging fluorescently labeled beads. The p-BNC analyzer’s fluorescent microscope illumination module consists of four equally spaced, obliquely oriented (45° from normal) blue LEDs (490 nm) that are filtered (482.5 / 31 nm) and relayed to the p-BNC chip via aspheric condenser lenses with a diffuser surface. The compact lens assembly consists of an objective lens (4×, 0.13 NA), emission filter (534.5 / 43 nm), and tube lens (100 mm). A 2/3” format monochrome CCD camera is attached to the tube lens. The optics module is mounted to a linear motorized stage that translates the entire assembly for focusing. An autofocusing algorithm was developed that works by translating to the vertical position with maximum variance in a region of interest containing the focal aid beads.

**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 such as the annular region of interest (ROI) method. The Gabor annulus method, based on the advantageous properties of Gabor wavelet filters, is a popular method for recognizing patterns.\(^{40}\) This 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.

**Fig. 5** shows the major steps of the image analysis process that is applied to each bead individually. First, the derivative of the raw image is used to isolate a bead’s edge from the background and bead interior. Then, multiple Gabor annuli are formed with slight variations in radius, frequency, and standard deviation. Using a range of Gabor annulus filters provides broader coverage for recognizing underlying patterns in the image\(^{31}\); here, the wavelet properties are tuned to account for the size distribution of beads. These filters are then convolved across the image, and the convolution responses of the filters 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, we developed a regional pixel analysis method called the annular ROI. Instead of averaging the pixels of the entire bead, the annular ROI method excludes low signal pixels in the bead center and focuses on the pixels that form the signal around the edges of the bead. Once the ROI is mapped, mean signal is extracted for each bead and a two-sided Grubbs’ test for outliers is applied using a significance level of \(\alpha = 0.10\).

**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 back 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 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. It also reduces background sources of noise by preventing stray light from entering the analyzer.

**Device operation**

With the bead sensors pre-loaded into the cartridge, the user deposits 2 drops (≥ 100 µL) 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 µL to be used in the assay. The user then inserts the card into the analyzer and starts the automated assay in which the remaining steps are performed automatically by the portable analyzer. The first step of the immunoassay delivers the sample to the bead sensor array. The right blister actuator steps down into the cartridge until a FSR detects the top of the blister. Then, the blister actuator continues to compress 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 µL/min) to allow adequate binding between antibodies and sample antigen. Wash steps are performed at faster flow rates (100-250 µL/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.

**Multifunctional tool for clinical and bioscience measurements**

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 p-BNC system’s multifunctionality, a series of in development assays were run using human serum samples covering three distinct disease applications: prostate cancer, ovarian cancer, and AMI. Fig. 6 shows data collected for each disease area. The prostate cancer panel (Fig. 6A) 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 (Fig. 6B), HE4 exhibits substantially higher signal for the late-stage ovarian cancer patient versus the healthy control while showing slightly lower signal in CA-125. This example highlights the importance of multiplexed panels in the diagnosis of ovarian cancer. While CA-125 is considered the gold-standard marker for prognosing ovarian cancer, a single-marker CA-125 test may have reported a false negative result for this late-stage ovarian cancer patient. In the four-plex cardiac panel (Fig. 6C), 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. 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 new alternative disease indications.

**Fig. 6.** Multi-functionality of the p-BNC system showing data obtained with injection molded cartridges (I) and portable analyzer prototype for three clinical applications: prostate cancer screening (A), ovarian cancer screening (B), AMI diagnosis (C). Fluorescent photomicrographs in II and III 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: CA-125 (red) and HE4 (green); four for the cardiac panel: cTnl (red), CK-MB (green), MYO (blue), and NT-proBNP (yellow). Mean fluorescence intensities (III.) were calculated via the annular ROI method.

**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. Serum myoglobin levels may be elevated within 1 to 2 hours of myocardial death while other markers like CK-MB and cTnl appear 3 to 6 hours after symptom onset; however, myoglobin has low specificity for cardiac necrosis, necessitating the inclusion of more specific cardiac biomarkers in the final cardiac panel. The characterization of the full cardiac panel will be presented in future work. Here, we developed a rapid (~7 minutes) myoglobin test on the p-BNC system. **Fig. 7** shows the results of the assay characterization. The dose response for myoglobin shows an adequate fit to the 5-parameter logistic curve ($R^2 = 0.9967$) with detection limit of 4.94 ng/mL (three standard deviations above
background signal, with background defined as the signal derived from two replicate blank runs. Yet to be fully optimized initial assay precision estimates were determined from five replicate runs. Inter-assay precision (%CV) for the positive calibrator, negative calibrator, myoglobin, and CRP sensors was 6.99, 12.05, 12.07, and 8.91%, respectively. Although preliminary and in need of correlation with reference methods, these assay characteristics suggest that laboratory-quality measurements could be obtained with the low-cost, portable p-BNC system in less time (7 minutes) than gold-standard reference methods (hours to days).

**Fig. 7.** Rapid myoglobin assay characterization. The dose response curve (left) shows the MFI at various dilutions (blue circles), intra-assay standard deviation from 4-fold bead sensor redundancy (error bars), and the calculated LOD (red “X”). The precision bar plot (right) 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).

**Conclusion**

Here we report the development of a portable, cost-effective, and sensitive detection system that is capable of remote multiplexed and multiclass clinical testing via standardized diagnostic test ensembles that can be quickly customized for new bioscience research and clinical applications. The p-BNC cartridge has successfully transitioned from a prototype device produced in modest volumes to a device designed for mass manufacturing. Further, we designed a portable analyzer intended for non-expert users which fully automates otherwise complex assay protocols, fluid motivation, optical detection, and data analysis. In this report, we have demonstrated the p-BNC’s versatility by quantifying 8 analytes across three different disease applications. Although preliminary and in need of further characterization, the p-BNC system has the potential to provide quality multiplexed and multi-class POC measurements for a variety of disease applications.

**Conflicts of interest**

Principal Investigator, John T. McDevitt, has an equity interest in SensoDX, LLC. and also serves on the Scientific Advisory Board. The terms of this arrangement have been reviewed and approved by Rice University in accordance with its conflict of interest policies.
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Notes and references

The programmable bio-nano-chip (p-BNC) is an ultra-flexible system for multiplexed and multiclass assays on a universal modular lab-on-a-chip platform for clinical and bioscience applications at the point-of-care.
Fig. S1. 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 our custom video analysis software. Volumetric flow rate was calculated by tracking the rate of fluid movement within this characterized structure.
**Fig. S2.** CAD drawing of the blister actuator module. This fluid delivery module consists of two linear actuators positioned over the p-BNC cartridge’s blister packs and supported by a 3-D printed plastic mount and carriage bolts. A cartridge alignment feature fastened to an acrylic base supports and aligns the card during actuation. The blister actuator module was used to validate the geometry-adjusted blister actuation method.
Fig. S3. CAD showing an alternate view of the portable analyzer prototype with the touchscreen, protective enclosure, and frame removed.
Fig. S4. CAD showing a perspective view of the disposable cartridge for cardiac testing.