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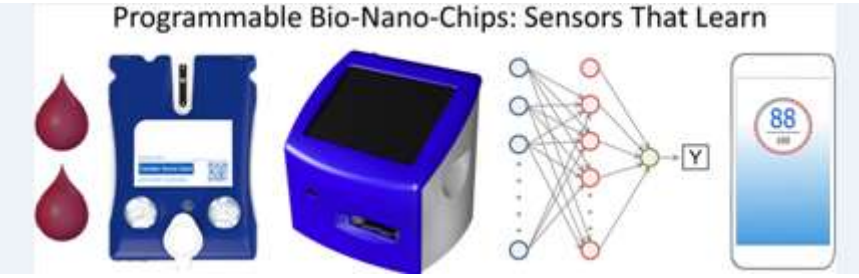
Programmable Bio-nano chip Platform: A Point-of-Care Biosensor System with the Capacity to Learn

Khitisweri Krupaya, Srinivas Mallick
Gandhi Institute of Excellent Technocrats, Bhubaneswar, India
Vedang Institute of Technology, Khordha, Odisha, India



CONSPECTUS: The combination of point-of-care (POC) medical microdevices and machine learning has the potentialtransform the practice of medicine. In this area, scalable lab-on-a-chip (LOC) devices have many advantages over standardlaboratory methods, including faster analysis, reduced cost, lower power consumption, and higher levels of integration andautomation. Despite significant advances in LOC technologies over the years, several remaining obstacles are preventing clinicalimplementation and market penetration of these novel medical microdevices. Similarly, while machine learning has seenexplosive growth in recent years and promises to shift the practice of medicine toward data-intensive and evidence-based decisionmaking,itsuptakehasbeenhinderedduetothelackofintegrationbetweenclinicalmeasurementsanddiseasedeterminations.In thisAccount, we describerecent developments in the programmablebio-nanochip (p-BNC) system,a biosensor platformwiththe capacity for learning. The p-BNC is a "platform to digitize biology" in which small quantities of patient sample generateimmunofluorescent signal on agarose bead sensors that is optically extracted and converted to antigen concentrations. Theplatformcomprisesdisposablemicrofluidiccartridges,aportableanalyzer,automateddataanalysissoftware,andintuitivemobile healthinterfaces.Thesingle-usecartridgesarefullyintegrated,self-containedmicrofluidicdevicescontainingaqueousbuffersconvenientlyembeddedforPOCuse.Anovelfluiddeliverymethodwasdevelopedtoprovideaccurateandrepeatableflowratesvia actuation of the cartridge's blister packs. A portable analyzer instrument was designed to integrate fluid delivery, opticaldetection, image analysis, and user interface, representing a universal system for acquiring, processing, and managing clinical datawhileovercomingmanyofthechallenges facingthewidespread clinicaladoptionofLOCtechnologies. Wedemonstratethep-BNC's flexibility through the completion of multiplex assays within the single-use disposable cartridges for three clinicalapplications:prostatecancer,ovariancancer,andacutemyocardialinfarction.
Toward the goal of creating "sensors that learn", we have developed and describe here the Cardiac ScoreCard, a clinical decisionsupport system for a spectrum of cardiovascular disease. The Cardiac ScoreCard approach comprises a comprehensive biomarkerpanelandriskfactorinformationinapredictivemodelcapableofassessingearlyriskandlate-stagediseaseprogressionforheart attack and heart failure patients. These marker-driven tests have the potential to radically reduce costs, decrease wait times, andintroducenew optionsfor patientsneeding regularhealth monitoring.Further,these eff orts demonstratethe clinical utilityoffusingdatafrominformation-richbiomarkersandtheInternetofThings(IoT)usingpredictiveanalyticstogeneratesingle-indexassessments for wellness/illness status. By promoting disease prevention and personalized wellness management, tools of thisnaturehavethepotentialtoimprovehealthcareexponentially.

INTRODUCTION

Weareenteringaneweraofchem-andbiosensingempowered byexponentialadvancesoccurringinanumberofdisciplines.WhiletheInternetageledtotheinterconnectednessbetween ment through the Internet of Things (IoT). EXtending thesetransformative changes to health care has the potential toexponentially improve lives. Despite the ubiquity of physicalsilicontransducersinvariousmobiledevicestoday,thereisa

peopleatanunprecedentedrate,thenextrevolutionwillinvolve _____ theconnectednessofobjects:integratingelectronics,computing,communications,andtransducerstocreateasmartenviron-lackofmobilehealth(mHealth)biomarkermeasurementplatforms that are programmable (i.e., can be easily retasked fora variety of applications) and accessible to individuals, chemists,pharmaceuticalscientists,andcare-providers,alike.Whilenearly70%ofcurrentmedicaldecisionsaremadeusingdiagnostic tests,[1]these tests for the most part are currentlyperformed in traditional healthcare settings using phleboto-mists, remote laboratories, delayed reporting, and an ineffi cientworkflow that stifles the arrival of novel biosensor technologieswith thecapacityto transform clinicaltestingand medicaldecisionmaking.

Microfluidic and lab-on-a-chip (LOC) systems are strongcandidates for providing the necessary "hardware" for thesechem- and biosensors. Originally inspired by microfabricationtechniquesfromthemicroelectronicsindustry,[2,3]LOCapproacheshavemadetheirwayintoseveralapplica tionssince their introduction in the early 1990s,[4]finding utility inmedicine,inkjetprinters,separationsciences,foodsafety,military,andveterinarymarkets.Arguably,point-of-care(POC)diagnosticsisthemostpromisingapplicationforLOCtechnology,wherescalablemedicalmicrodevicesoff erfaster analysistimes,reducedvolumesofbioreagents,lowerpower requirements, and higher levels of integration and automationthanstandardcentralandremotelaboratorymethods.[5]However,despitethe potentiallyenormous societalimpact ofLOC technology, major barriers are preventing the translationof these novel systems from the laboratory to routine clinicalpractice, such as lack of integration and failure to compete withbothperformanceandcostoflaboratory-basedtests.[6−8]Further, the field is currently experiencing significant challengesassociatedwithoverlyaggressiveprojectionsofcurrentcapabilitiesofselectedeff orts.Despitethesechallenges,thereis greatopportunityforLOCtechnologiesprovidedthatopen and honest evaluations comprising extensive clinical validationand peer-reviewed reports are made widely available to clinical,regulatory,commercial,andgeneralpublicaudiences.

Similarly, machine learning has seen explosive growth inrecentyearsduetotheemergenceofnewdataminingtechniques,theincreasingavailabilityofdata,andthedecreasing cost of computation.[9]This widespread adoption ofartificial intelligence (AI) systems over the past two decades hasresulted in a paradigm shift toward data-intensive and evidence-baseddecisionmaking,spanningavarietyofdisciplinesincluding chemistry across government, industry, and academicinstitutions,alike.Likewise,machinelearningisplayinganincreasingly important role in chem- and biosensingapplica-tions and within the practice of medicine; however, despiteenormoustechnologicalprogress,severalchallengesarepreventing AI systems from reaching their full potential.[10]Inthe context of clinical laboratory measurements, one of theprimarybarriersisthelackofintegrationbetweendataacquisition, handling, and interpretation. There is an oppor-tunity for universal and AI biosensor systems to significantlyimprove health care by acquiring, processing, and managingclinicaldata.

InthisAccount,wedescribeourmostrecentworktoward developing the programmable bio-nanochip (p-BNC) ensem-blewiththecapacitytolearn.[11−16]Thismultiplexandmulticlass platform for bio- and chemical analysis has beendemonstrated previously in its ability to assess disease/healthstatusinoralcancer,ovariancancer,prostatecancer,cardiacheart disease, and trauma using over 22 protein biomarkers, 12smallmolecules,and13cellularmarkers.[17]Thep-BNCsystem shown in Figure 1is a flexible platform for digitizing biology,featuringsensorensemblesthatmeasurebiomarkersinahighly

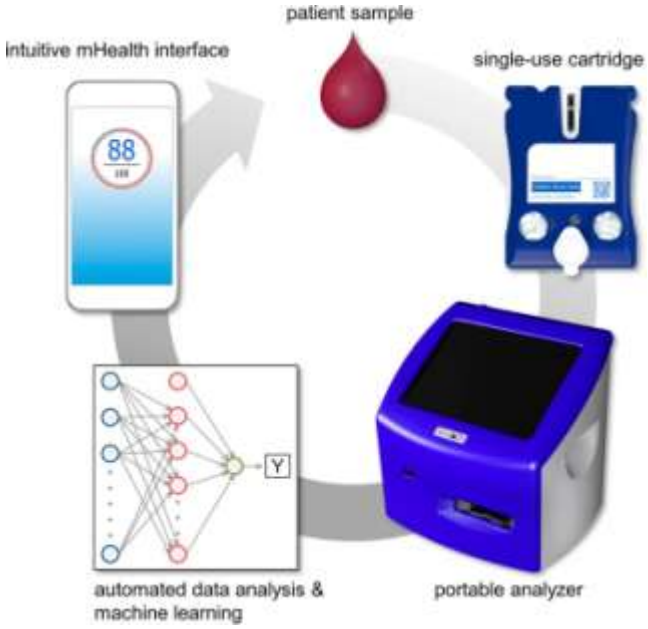


Figure 1.Intended use cycle of the p-BNC system for routine cardiacwellnesstesting.

efficient manner. The process begins by introducing a smallquantity (~100 µL, 2 drops) of patient sample (e.g., serum,plasma,ororalfluids)intoadisposablecartridge.Thecartridgeisinsertedintotheportableanalyzer,whichautomatically performs the multistep assay sequence. Image analysis routines"digitizebiology" byconvertingthesignalintobiomarkerconcentrations. The biomarker concentration data then flowsintodisease-specificmachine-learningalgorithmsthathavebeen trained on >1000 patient clinical trials to predict aspectrum of cardiovascular disease (CVD). The result is asingle value "Cardiac Score", which is then displayed to thepatientusinganmHealthapp.Providingpatientswithpersonalized wellness information has the potential to promoteprevention and active management of cardiac health, and thecombination of high-sensitivity POC diagnostics and machinelearning has the potential to transform health care movingforward. While this Account exclusively highlights the p-BNCsystem, more comprehensive reviews of the LOC field as awholehavebeenpublishedbyothergroups.[2,5,7,18]

SINGLE-USEMICROFLUIDICCARTRIDGES

Criticaltothe"sensorsthatlearn" conceptarethesensorensembles themselves and the platform technology that enablesprotein,antibody,smallmolecule,andoligonucleotidebiomarker measurements at the POC. To illustrate how thep-BNC technology functions, Figure 2depicts the bead-basedassaysystemacrossvariouslengthscales.Thep-BNCcartridgeisafullyintegrated,self-containedmicrofluidicdevicethathas aqueousbuff ersconvenientlyembeddedforuseatthePOC.Inthecurrentconfiguration,thisinjection-moldedcartridgecontainsa4× 5matrixofflow-throughmicrocontainersdesigned to hold agarose bead sensors. Multiplexing is achievedthrough spatially programming the bead sensors within themicrochipwherequantitationofproteinsandantibodiesoutperformsELISA,achievinglowerlimitsofdetection,faster

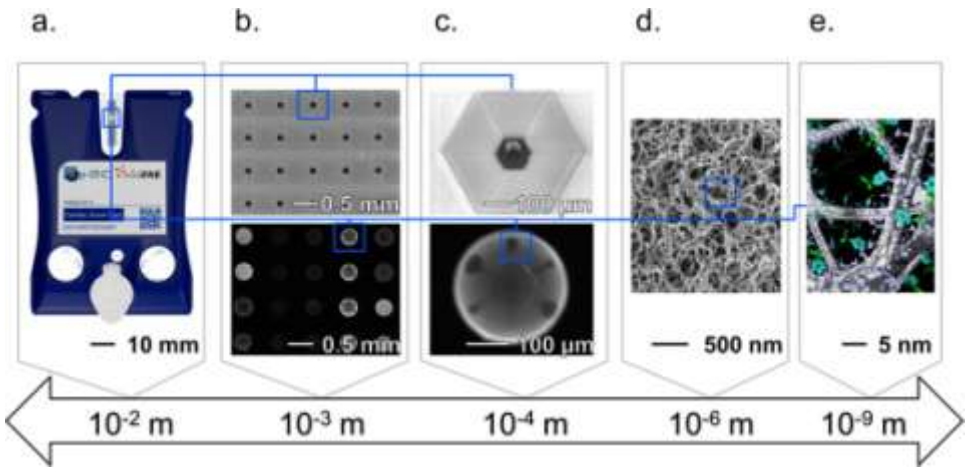
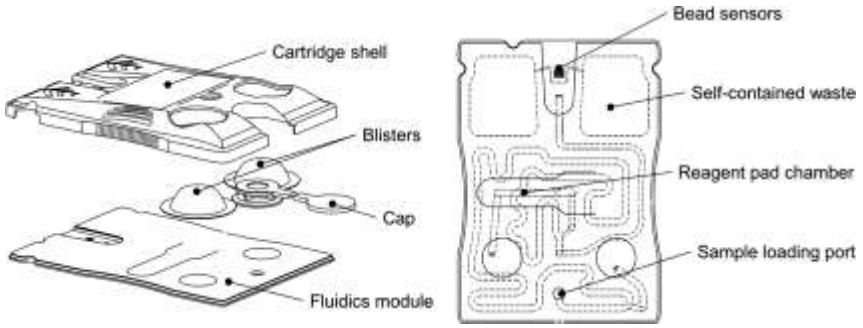


Figure 2. A graphical depiction of p-BNC features at various length scales: (a) The approximately credit-card sized p-BNC cartridge. (b) The p-BNC's 4 × 5 bead array with (bottom, fluorescence image) and without (top, SEM image) agarose bead sensors. (c) Magnified view of a single beadsensor (bottom,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



(e)Illustrationofatypicalsandwichimmunoassayperformedonanagarosebeadfiber.

Figure 3.Illustrations of the p-BNC cartridge. The exploded diagram (left) reveals the main fluidics module onto which two reagent blister packsandanadhesivecaparemounted.Thecartridgeoutershellenclosesandprotectsthecartridge.Thefluidroutingdiagram(right)showsthelocationof the sample entry, user-configurable reagent pad chamber, self-containing waste chambers, and bead sensor array. Reproduced from ref 15 withpermissionfromTheRoyalSocietyofChemistry.

analysis times, and improved usability.[13]The six-sided flow-throughmicrocontainerdesignallowsconvectivetransporttopenetrate the agarose beads, where short depletion layersimprovecaptureefficiencyrelativeto2-Dmethods.[19]Theagarose beads' 3-D fibrous network that is indexed matched towater provides a distinct advantage over 2-D capture (e.g.,lateral flow devices, planar microarrays, 96-well plates) becausethe3-Dlatticestructureconcentratesahigherdensityofimmunocomplexes within the optical path of the fluorescencemicroscope,resultinginhighersignaland,thus,improved sensitivity.Otheradvantagesofthisagarosebeadsysteminclude(1)drastically reducedanalysistimes relativetodiffusion-dominant ELISA, (2) the ability accommodate awide range of target analyte properties (e.g., molecular weight,size,and shape)bycustomizingporosity,(3)a capacitytoperform both two-site immunometric and competitive assayformats,and(4)lownonspecificbindingcharacteristicsprovided by this polymerized sugar matrix. The first p-BNCdesigns were composed of anisotropically etched silicon 100wafers. Recent efforts to translate these devices into clinicalpractice have resulted in the mass-produced, globally scalable,inexpensiveinjectionmoldedplasticcartridgesshownhere.

A common obstacle for translating LOC devices from R&Dlaboratoriestoclinicalpracticeistheabsenceofrobustinterfacesforthechipanditsperipheralreagents.These complicated"world-to-chip"interfacesrendersomeLOCdevices impractical outside of research settings, inheriting thetitle "chips in a lab".[6,7]The methods through which fluids (e.g.,the specimen, bioreagents, aqueous buffers, and waste) aremanipulated must be carefully considered when designing amicrofluidic device. Further, successful LOC devices minimizemanual fluid handling steps and perform preprocessing stepswithinthedevice.

The p-BNC microfluidic cartridge (Figure 3) streamlinesvariousfluidhandlingsteps.First,asmallvolumeofbiospecimen(i.e.,serum,plasma,urine,ororalfluids)isintroduced into the loading port. The sample fills the fluidiccartridgeviacapillaryflow,andapassivevalvegovernsautomatic metering(100 μL) of sample volumes.After thesample is loaded, the user closes the device via an adhesive cap."On-chip" processing of sample is completed by inline filters.Thecartridgefeaturestwofoilblisterpackscontainingphosphate-buffered saline(PBS),whichserveastheaqueous buffers. Puncturing mechanisms on the cartridge underneaththe blisters rupture the foil upon external compression via theanalyzer's automated blister actuation system. The right blistercontrols the antigen delivery pathway while the left blistercontrols the detecting antibody pathway. An easily accessiblereagent pad chamber on the top face of the cartridge holds aglassfiberconjugatepadwithdetectingantibodyreagentsthat

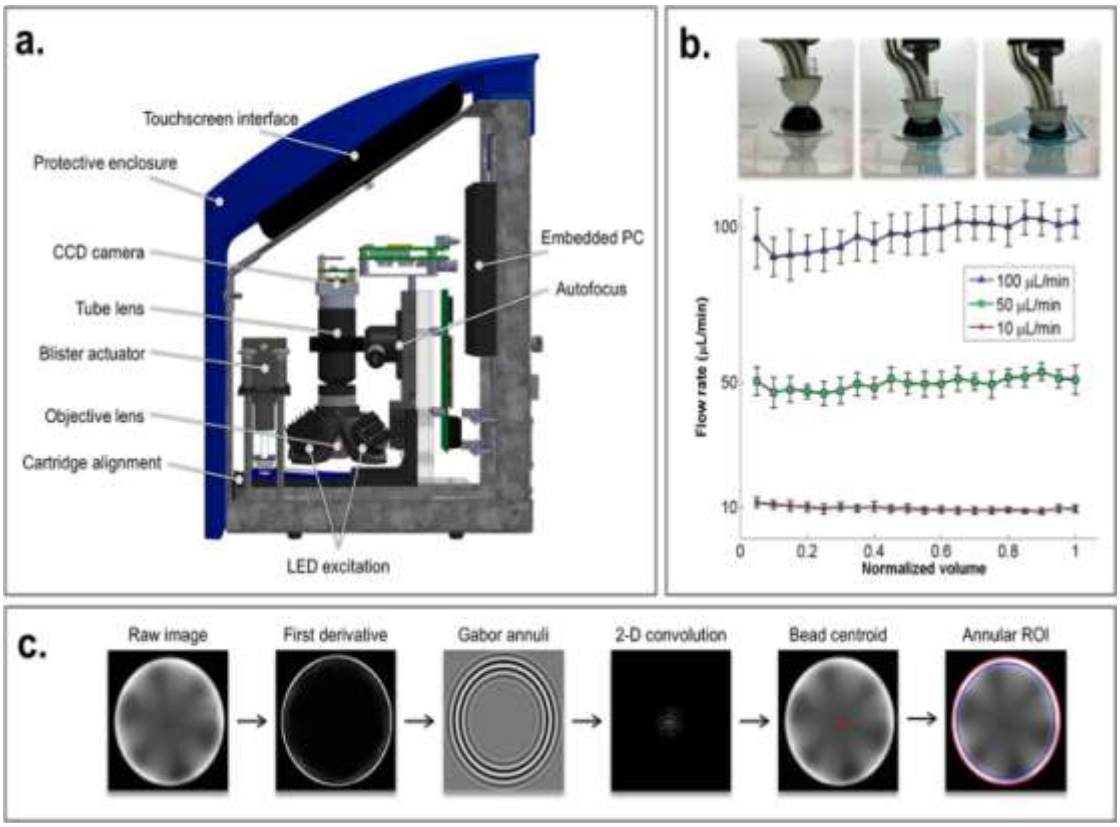


Figure4.Portableandautomatedinstrumentationforthep-BNC.(a)Illustrationoftheanalyzerprototype(internalsideview).(b)Automatedfluiddeliverysystemshowingimagesoftheactuatorcompressingablisteratvariousheights(top)andflowrateverificationresults(bottom)withmeanflow rates and standard deviation (error bars) for five runs at each target flow rate. (c) Summary of the automated image analysis procedure, whichlocates,extracts,andaveragesthebeads'fluorescencesignal.Reproducedfromref15withpermissionfromTheRoyalSocietyofChemistry.

are eluted and delivered to the bead sensor array. Any unboundreagents and antigen are drained into waste chambers adjacentto the bead array. When the assay is complete, the cartridgemay be removed from the instrument and safely disposed in abiohazardouswastecontainer.Thep-BNC'shighleveloffluidicintegrationreducespotentialhumanerrors,improvesrepeat-ability, and qualifies its use at the POC. Additionally, theautomation of fluid handling steps allows p-BNC assays to beperformedwithminimaltraining.

PORTABLE AND AUTOMATED POINT-OF-CARE ANALYZER

Integratingmicrofluidicdisposablesandtheirassociatedinstrumentationintoaunifiedanalysissystemis aprimarychallengeforPOCdiagnostics.[20]Inthecaseoffluorescenceimmunoassays,theopticaldetectionsystemmustbeportable,inexpensive,andsensitive.Recentimprovementsinoptoelec-tronicshardwarelikeCMOScamerasandLEDsoff ernewlow-costandsensitiveoptionsforLOCinstrumentation.Similarly,astheperformancecharacteristicsofsingle-boardcomputersforembeddedsystemscontinuetoimprove,increasinglysophisticatedsoftwareprovidesnewopportunitiesfordevel-opersto improve device performanceanduser experience.Thegoalfordevelopingasuccessfulportableassaysystemistocreateanautomatedworkflowthatrequiresnospecialtraining.Thep-BNCanalyzerprototypedescribedpreviously[15]andshowninFigure4isapproximately22cm×22cm×30cm(l × w× h),weighslessthan7kg,andfeaturesacompact fluorescenceimagingmodule,automatedfluiddeliverysystem, cartridgealignment,embeddedPC,andtouchscreeninterface(Figure4a).Theanalyzer'sminiaturizedopticalsystemisdesigned to image fluorescently labeled beads (AlexaFluor-488)and consists of an off -axis illumination module with four blueLEDs that are filtered (483 nm) and directed to the objectplanewithaspheric condenserlenses.Theinlineopticalassemblycomprisesanobjectivelens(4×,0.13NA),anemission bandpassfilter (535 nm), a tube lens, and a CCDimager attached to a precision positioning actuator for focusing.Inadditiontothecompactfluorescencemicroscope,anautomatedfluiddeliverysystemcomprisingtwoverticallyorientedactuators compressesthe cartridge's blisterpacks toperformcustomizableflowprotocols.Thecartridgeslotcontains a

loading mechanism that aligns the cartridge withthe blister actuators and optics field of view. The p-BNCsoftware user interface is displayed and manipulated via aninteractivetouchscreen.

Themethodofmotivating fluidswithinamicrofluidic device

is an important consideration when developing a new deviceconcept. While there are a variety of driving forces to choosefrom, blister packs are a popular choice for POC settingsbecause the aqueous buff ers can be stored conveniently on thedevice. Several groups developing POC devices have selectedblisterpackactuationastheirprimarysourceoffluidmotivation[21]including commercial products such as the Abbotti-STAT device[22]and the Daktari Diagnostics device.[23]Despitethe numerous examples of blister pack actuation systems in theliterature,littleinformationregardingtheaccuracyandprecisionoftheflowratesresultingfromactuationhavebeen

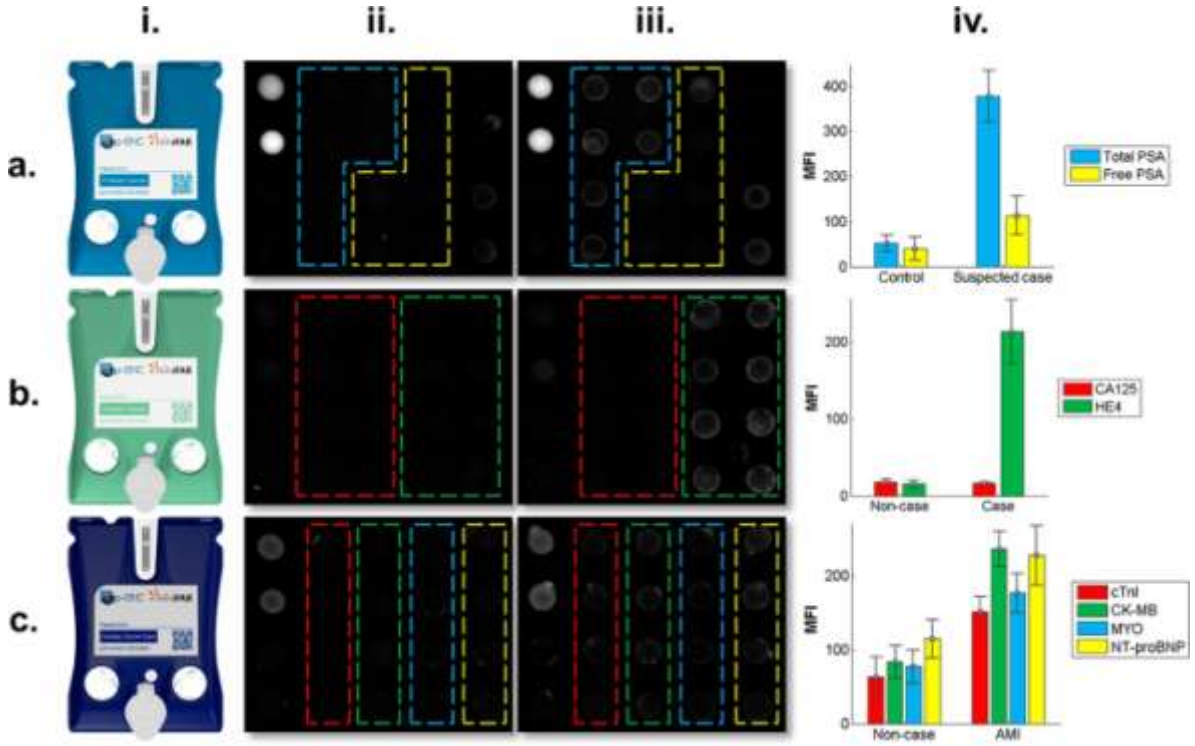


Figure 5.p-BNC'smultifunctionality was demonstrated by completing assays within the single-use disposable cartridges (i). Fluorescence images areshownfornoncases/controls(ii)andcases(iii)whereeachpanelincludespositivecalibratorsandnegativecontrolsattheleft-mostcolumnofthebead array. Analytes were detected for each disease application; total PSA (blue) and free PSA (yellow) for prostate cancer (a); CA125 (red) andHE4(green)forovariancancer(b);cTnI(red),CK-MB(green),MYO(blue),andNT-proBNP(yellow)forAMIdiagnosis(c).Meanfluorescenceintensities(iv)weredeterminedfromtheannularROImethodwitherrorbarsshowingthestandarddeviationfromduplicatebeadsensorsfromasingleexperiment.Reproducedfromref15with permissionfromTheRoyalSocietyofChemistry.

published. Previously, our group showed that flow rate is animportant parameter for optimizing analyte capture;[19]there-fore, delivering accurate and repeatable flow rates was a highprioritywhendesigninginstrumentation.

Recently,wedevelopedamethodfordeliveringhighlycustomizable and repeatable flow rates with blister actuation.[15]Our fluid delivery system features two key innovations: blisterburst detection and a dynamic blister actuation model. Forcesensitive resistors are embedded underneath the blister actuatortips, and a burst detection algorithm recognizes the event whenthe blister has ruptured, allowing the automated fluid deliverysystem to track and administer accurate flow rates into thecartridge. Once the blister is burst, the actuators compress theblister with an actuation rate derived from a geometric model ofthe blister volume. The blister was mounted to an experimentalmicrofluidiccardwithknownvolume,andvideoanalysissoftware estimated the volumetric flow rate by measuring thevelocityofadyesolution(Figure4b).Flowratesatthreetargetlevels (100, 50, and 10 μL/min) were delivered for five runseach, demonstrating exceptional control of fluid delivery. Theautomationofsampleandreagentfluidseliminateslabor-intensivemanipulationsandimprovesrepeatabilityandreproducibility en route to making quality standard measure-mentsatthePOCareality.

Automateddataanalysisroutinesthatwouldpreviouslybeconsideredtoocomputationallyintensiveforportable POCsystemsaren

owfeasibleassingle-boardcomputersarebecomingbetter,faster,andcheaper.Inthismanner,increasinglysophisticatedsoftwaresolutionsareexpectedtorelax,andperhapsdisplacealtogether,certaindesignrequirements resulting in LOC systems that are easier and cheaper tobuild. In addition to algorithmically controlling fluid delivery,thep-BNCanalyzer automatestheanalysisof fluorescencemicrographs,convertingpixelintensitiesintobiomarkerconcentrations.[15]The image analysis software summarized inFigure 4c and applied to each bead individually uses novelcomputer vision methods to detect the bead's locations andanalyze pixels within a region of interest (ROI). First, the finitediff erence image identifies the bead's outer edge. Next, beadswithintheexpectedsizedistributionareidentifiedviageneratingseveralGaborannuli[24]withslightlydiff erentpropertiesandsubsequentlyperforming2-Dconvolutionoperations on the derivative image. The resulting convolutionresponses are normalized and averaged, and the bead locationsare defined by the maximum averaged response. The outer edgeis determined by fitting a circle to the points of maximumintensityin thefinitediff erenceimage.Next,anannularROIis mappedalongthebead'souteredge,thesignalwithintheROIis averaged, and a Grubbs' test removes outlier beads from theanalysis.Lastly,theresultingmeanfluorescentintensities (MFI)areconvertedtobiomarkerconcentrationsbyreferencingapredetermineddosecurve(four-orfive-parameterlogisticcurve).

To demonstrate the p-BNC system's bioassay flexibility, aproofofconceptstudywasperformedusingtheintegratedand automatedbioassayplatformdescribedpreviously.[15]Indevelopment assays for the screenings of prostate and ovariancancer and acute myocardial infarction (AMI) diagnosis wereconducted on the p-BNC system using human serum samplesforcasesandcontrols(Figure5).Theprostatecancerscreening

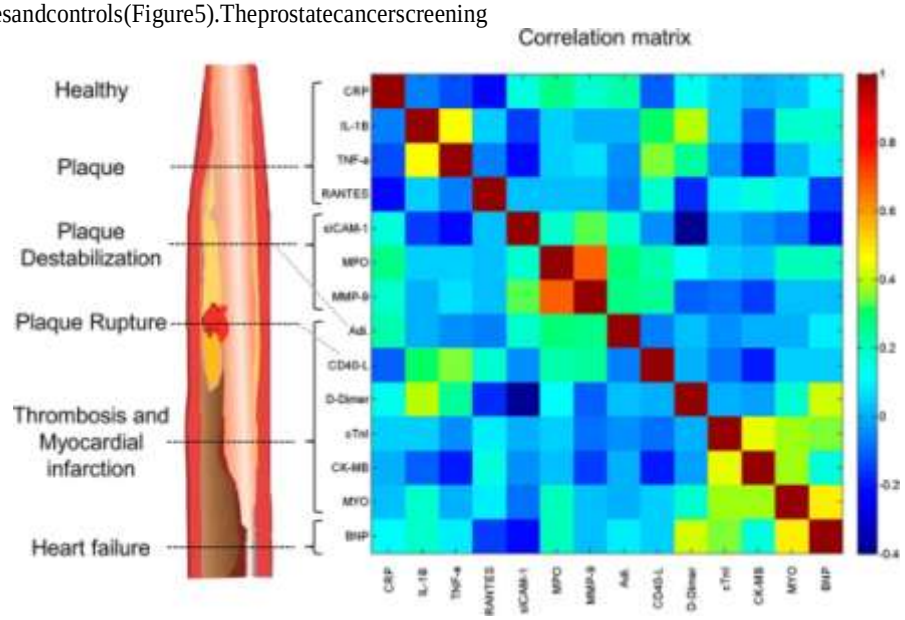


Figure6.Cardiacbiomarkersandtheirpathophysiologicalroleinatherosclerotic plaquedevelopment.Anidealizeddiagram(left)showssimplifiedstagesofatherosclerotic plaquedevelopmentandassociatedbiomarkers.Acorrelationmatrix(right)showsthePearsoncorrelationcoefficientmatrixfor allpatients(N=579)andallpatientoutcomesinthestudy.Reproducedwithpermissionfromref16.Copyright2016Elsevier.

panel (Figure 5a) exhibits higher signal in both the total andfree PSA beads for the suspected positive case, indicating thatthe p-BNC can distinguish between prostate cancer cases andcontrols. Similarly for ovarian cancer, the p-BNC (Figure 5b)distinguishes a late-stage cancer patient from a noncase patientvia substantially higher signal in HE4 bead sensors. For AMIdiagnosis (Figure 5c), all four cardiac biomarkers (cTnI, CK-MB,MYO,andNT-proBNP)areelevatedintheAMIpatient,suggesting the p-BNC's utility in distinguishing between AMIandnoncasechestpainpatients.Thiscompilationofdatashowcasesthep-BNCplatform'sflexibilityinwhichauniversalcartridge can be reconfigured via the spatial programming ofbead sensors. While the work described here demonstrates thep-BNC's capacity for highly customizable multiplex panels tocoveranumberofdiseaseindications,ourpriorworkperformed on less integrated instrumentation enumerates theassay performances for a selection of analyte targets[17]anddedicated multiplex panels such as drugs of abuse,[14]cardiacheartdisease,[25]andovariancancer.[12]

CARDIACSCORECARD FORPREDICTINGASPECTRUMOFCARDIOVASCULARDISEASES

Theimplementationofmachineandstatisticallearningindiagnostic devices has the potential to radically alter the way wequantify our health, and once adopted clinically these devicescould outperform current standard methods for diagnosing anddeterminingprognosisofdiseases.[26]Notonlywillthecompositeinformationonmultiplexbiomarkerpanelsoutperformanysingle-markerdiagnostictest,butalsotheintroductionofnewdiseasescoresandclassificationsprovidesaresultthatismoreinterpretablethansimplyalistof biomarker concentration values. The bridge between integratedPOC testing and model development is critical for translatingtheoretical approaches into mainstream clinical practice. In thiscapacity,ourgroupisdevelopingtheCardiacScoreCard,statisticallearningmodelsthat predictcardiovasculardiseasesusing data from multiplex cardiac marker panels and risk factorinformation.[16]CVD is the leading cause of death in the U.S.andglobally,andtheexorbitanthealthcarecostattributedtoCVD is burdening the U.S. economy. The joint goal of savinglives and reducing healthcare costs may be accomplished byshifting the focus from late-stage disease maintenance to earlydetectionandpreventionofCVD.

Clinical decision support systems (CDSSs) are support toolsthat assist in medical decisions by providing clinicians withpersonalized assessments or recommendations[27]and off er apromising solution for managing CVD. Several CDSSs forCVD have been developed, featuring various machine-learningmethods such as artificial neural networks,[28]Support VectorMachines,[29]randomforest,[30]Bayesiannetworks,[31]logisticregression,[32]andensemblemethods.[33]AlthoughCDSSs promiseenhanceddiagnosticresults,shorterwaittimes,andreduced cost versus the standard of care, physicians may behesitanttoimplement"blackbox"CDSSs(i.e.,thealgorithm's resultsandmethodstoobtainthemareeitheruninterpretable ornotcapableofprovidingactionabletherapeuticrecom-mendations). Therefore, the Cardiac ScoreCard uses a lassologisticregressionapproach,convertingriskfactorsandbiomarkerdataintoasinglescorewithinterpretableandclinically useful information in the form of logistic regressioncoeffi cients. When fully developed, the Cardiac ScoreCard isintendedtoprovidepersonalizedcardiachealthassessments

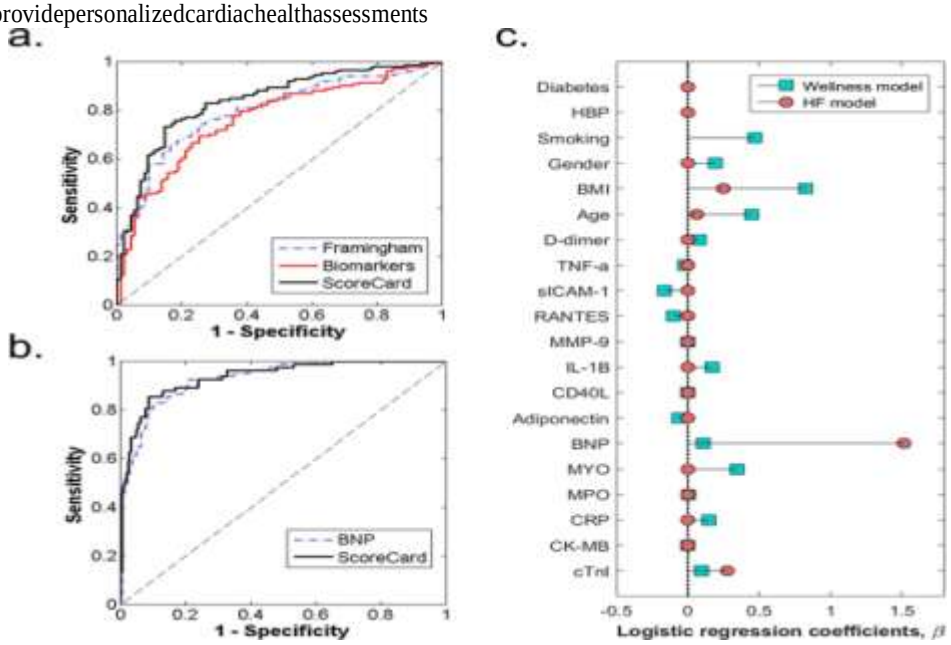


Figure 7.Results of the Cardiac ScoreCard and various reference methods. (a) Receiver operating characteristic (ROC) curves for the cardiacwellness application (high versus low risk) for the Framingham 10-year risk score, a biomarkers-only model, and the Cardiac ScoreCard. (b) ROCcurves for HF diagnosis using a single-marker BNP test and the Cardiac ScoreCard. (c) Lasso logistic regression coefficients for the CardiacScoreCardwellnessandHFmodels.Reproducedwithpermissionfromref16.Copyright2016Elsevier.

across a spectrum of CVD with predictive models for cardiacwellness, AMI diagnosis, AMI prognosis, heart failure (HF)diagnosis, and HF prognosis. In this Account, we report ourrecent results for cardiac wellness and HF diagnosis,[16]while theotherswillbefeaturedinfuturepublications.

The Cardiac ScoreCard assay comprises a multiplex panel ofbiomarkers from a diverse pathophysiology for two reasons(Figure 6). First, others have demonstrated that a multimarkerstrategy with pathobiologically diverse biomarkers improvesperformance in CVD risk prediction.[34]Further, a selection ofmarkers that are differentially expressed across various phases ofatherosclerotic plaque development provides new opportunitiesfor CVD prediction models. Second, it is important to train themodels with predictors that are uncorrelated. When biomarkersfrom the same pathophysiology are selected for a model, thepredictors tend to be highly correlated. Conversely, selectingbiomarkers from a diverse background are expected to increasetheoverallinformationcontentinthepredictivemodel.

A model for assessing and predicting overall heart health forconsumerwellnesstestingwasrecentlydevelopedanddescribed previously.[16]Briefly, the wellness ScoreCard model(AUC=0.84)outperformedboththeFramingham10-yearCVD risk score (AUC = 0.80) and a biomarker-only model(AUC = 0.77) in terms of discrimination between high risk andlowriskpatientgroups(Figure7a).Additionally,theScoreCardmodel showed good calibration across deciles of predicted risk(Hosmer−Lemeshowp=0.98),demonstratingitsusefulnessasascoreforwellness.Similarly,aHFdiagnosismodelwas developedandcomparedwithastandardmethodfordiagnosis

(BNP test). The multivariate ScoreCard algorithm showedbetter discrimination in diagnosing HF than the single-markerBNP score (AUC = 0.94 and 0.93, respectively) (Figure 7b),suggestingthatthediagnosisofHFcanbeenhancedbyaddingauxiliarybiomarkersandpatientdemographics.

Oneadvantageousfeatureoflassologisticregressionisautomatic feature selection.[35]Feature selection reduces modelcomplexity and improves generalization by discarding unneces-sary predictors while retaining the most relevant, and the lassologistic regression method performs this feature selection byshrinkingtheregressioncoefficients.Notonlydoesthisfeatureselection approach improve prediction performance, but it alsoprovides assay developers with a prioritized list of biomarkercandidatesforfutureimplementationinmultimarkerpanels.Figure7cshowsthelogisticregressioncoefficientsforboththeWellnessandHFmodels.Inthewellnessmodel,thelassomethod selected 15 predictors (i.e., nonzero coefficients) withBMI(β_{BMI}=0.82),smoking($\beta_{smoking}$=0.47),age(β_{age}=0.45),myoglobin(β_{MYO}=0.34),gender(β_{gender}=0.19),andIL-1β

($\beta_{IL\text{-}1\beta}$= 0.17) having the largest effect sizes. These resultssuggestthatthediscriminationbetweenhighandlowrisk patients is contingent on a relatively large number of predictors,comprisingdemographicsandbiomarkersfromadiversepathophysiology.On the other hand, the lasso method returneda sparse model for HF diagnosis with only four nonzeropredictors:BNP(β_{BNP}=1.51),cTnI(β_{cTnI}=0.28),BMI(β_{BMI} =0.25),andage(β_{age}=0.06).Fromapracticalassaydevelopment perspective, this sparse HF model, made possiblethroughlasso-basedfeatureselectionmethods,has thepotential

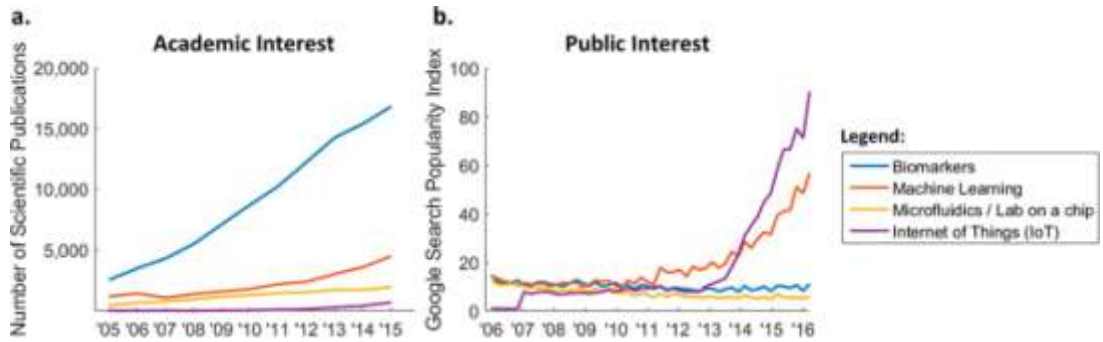


Figure8.Academicandpublicinterestsinvarioustopicsoverthepastdecade:(a)Numberofscientificpublicationsreportedbytheweb ofScience(http://www.webofscience.com). (b) Relative popularity of topics in the United States (total searches for the topic out of all Google searches)reportedbyGoogleTrends(http://www.google.com/trends).

to significantly reduce the cost of reagents and simplify theassaychemistry.
Theimportanceoftranslatingbiomarkerdataintointerpretable wellness scores is further underscored by thedisparity between academic and general public interests. In thepastdecade,over100 000biomarkerpapershavebeenpublished with the number of biomarker papers increasingevery year; however, the relative public interest in biomarkersappearstobedecliningovertime(Figure8).Tobridgethegap,researchers must convey biomarker information more eff ec-tively to the general population by developing intuitive healthreportcards,liketheCardiacScoreCard.TheCardiacScoreCardtechnologywillprovideacomprehensivebiomarkerpanel capable of assessing early risk as well as monitoring latestagediseaseprogressionforAMIandHFpatients.Thesemarker-driven tests have the potential to radically reduce costs,decrease wait times, and add new options for patients needingregular health monitoring. Expanding on the capabilities ofconsumerelectronics,bigdataanalytics,andweb-awaresensors,cloud-connecteddiagnosticscanbepowerfulinstru-ments for wellness tracking and behavior modification. Thefusionofdatafrominformation-richbiomarkersandIoTinfrastructureswithpredictiveanalyticsmayexponentiallyimprovedrugdiscoveryandhealthpolicyandallownewoptionsforpersonalizedwellnessmanagement.

CONCLUSIONS AND OUTLOOK

Thepotentcombinationofmedicalmicrodevices,newbiomarkermeasurements,andmachinelearninghasthepotential to transform medicine by empowering individuals toplay more active roles in the management of their own wellnessstatus. However, while the application of AI in medicine is stillin its infancy, several challenges remain for these integratedchem- and biosensing strategies to reach their full potential.With the ever-increasing value and volume of data, we will becontinually faced with ethical questions regarding the owner-ship and use of these sensitive data. Researchers developingnewCDSSsneedtoprioritizepatientconfidentialitybydesigning systems that provide secure user authentication anddata transactions. In addition to privacy concerns, issues withthe integration of data acquisition, handling, and interpretationcontinues to plague the healthcare industry. Integrated chem-and biosensor platform developers need to design universalsystems that are compatible with the workflows of healthcareprovidersinordertostreamlinedatatransactions.Lastly,cliniciansmaybereluctanttoadoptCDSSsthatuse“blackbox”
methods(e.g.,artificialneuralnetworksandSVMs)and

provideuninterpretable results. When developing machine-learning algorithms, researchers should implement models thatprovide both interpretable rationale for diagnostic decisionsandtheessentialinformationtomakepatient-specificrecommendations. The combination of a platform to digitizebiology and predictive analytics can change the trajectory ofmedicine, where the current linear thinking, mainly based onlate-stagediseasediagnosisusingexpensiveandcumbersometools, is replaced by a pathway to exponential medicine madepossible through the introduction of scalable tools with thecapacitytolearn.

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