# Versatile Low-cost Modular Microfluidic Arrays for Cancer Diagnostics

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Abstract: Accurate, sensitive, multiplexed detection of biomarker proteins in serum and tissue holds significant promise for personalized cancer diagnostics and therapy monitoring. Here we describe fabrication details of a modular microfluidic system featuring a small chamber for on-line protein capture from serum by magnetic beads, positioned upstream of a nanostructured multi-sensor array chamber to achieve high sensitivity for up to eight proteins, with the ability to expand to many more proteins. Microfluidic chambers are made by templating PDMS channels on machined aluminum molds to avoid lithography, and mounted in hard plastic housings equipped with inlet and outlet lines and interfaced with valves. Gold immunoarrays fabricated by screen or ink-jet printing, or wet chemical etching of gold films utilize amperometry or electrochemiluminescence (ECL) detection. These arrays are interfaced with microfluidics to achieve well-controlled mass transport leading to excellent signal/noise and unprecedented sensitivities. With interest in low cost point of care (POC) systems, we developed a module to also facilitate automated microfluidic reagent and sample delivery utilizing an open source microcontroller and micropumps, with ECL detection by camera.

## 1 INTRODUCTION

Measuring diagnostic panels of multiple proteins in serum holds great promise for future personalized cancer screening and therapy monitoring (Manne, Srivastava and Srivastava, 2005) (Rusling, Kumar and Gutkind, et al. 2010) (Ludwig and Weinstein, 2005) (Rusling, 2012) (Rusling, Munge, et al. 2013). It is necessary to measure multiple proteins in samples from each patient because a single protein biomarker is subject to too much individual variability to give highly accurate predictions. Thus, the potential to measure concentrations of panels of biomarker proteins for cancer diagnostics has created great interest in the biomedical community for some time (Kulasingam and Diamandis, 2008). (Hanash, Pitteri, and Faca 2008) (Giljohan and Mirkin, 2009) (Hanash, Baik and Kallioniemi, 2011). Unfortunately, broad realization of such diagnostic strategies has yet to be achieved. This is due in a large part to the lack of suitable inexpensive, sensitive devices to measure multiple biomarker proteins in patient samples, as well as the lack of fully validated panels for cancer diagnosis. For clinical or point-of-care (POC) use, technical simplicity of protocols and low cost are essential. Emerging aspects of nanotechnology and materials science combined with microfluidics provide exciting new opportunities to design and fabricate such devices (Rusling, Munge, et al. 2013). This paper will describe development of prototype modular microfluidic systems capable of ultrasensitive detection of multiple serum proteins (Figure 1). Unlike our previous publications describing multiplexed protein detection with some of these systems, the present paper will focus on fabrication, system optimization, and new automation aspects of the devices.



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## 2 SYSTEMS FOR PROTEIN DETECTION

For protein detection, a 100 µL modular cylindrical chamber for on-line protein capture from serum on magnetic beads is positioned upstream of a nanostructured multi-sensor array in a 60 µL chamber to achieve high sensitivity for up to eight proteins (Figures 1 and 2) (Otieno et al., 2014) with the ability to expand to many more proteins. The microfluidic chambers are made by templating PDMS channels on machined aluminum molds to avoid lithography. The PDMS slabs are mounted in hard plastic housings equipped with inlet and outlet lines to construct the desired chambers, and interfaced with valves. These modular microfluidic immunoarrays utilizing amperometry or electrochemiluminescence (ECL) detection chambers interfaced with microfluidics achieved well-controlled mass transport leading to excellent signal/noise and unprecedented sensitivity.

Amperometric multi-sensor array chips were fabricated by ink-jet printing of 4 nm alkylthiol gold nanoparticles (€0.20/chip) (Krause, et al., 2013) commercial screen printing of carbon and coating with 5 nm glutathione-gold nanoparticles (€7/chip) (Chikkaveeraiah, et al., 2011), wet-etching to fabricate gold CD arrays (Tang, et al., 2012), or, for ECL, microwell-patterning of pyrolytic graphite chips (€0.20/chip) (Sardesai, et al., 2013). A simple print/heat/peel method was developed to transfer computer printed patterns onto the arrays to create hydrophobic wells as small as 10 nL around each sensor. Briefly, a toner pattern is printed by a laser jet onto high gloss paper, and transferred onto the array substrate in a heat press. These toner nanowells can hold 1 µL solutions to facilitate building nanostructures and attaching antibodies on sensor elements while avoiding cross-contamination.

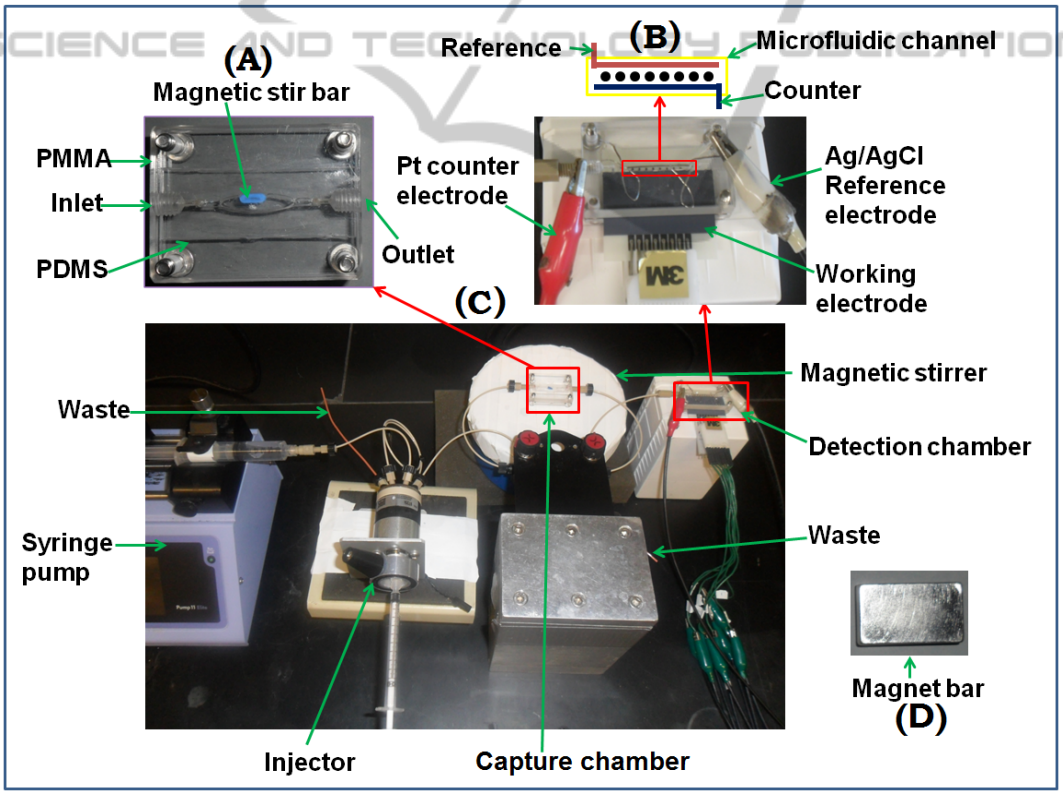


Figure 1: Prototype modular microfluidic system for on-line protein capture and amperometric detection using magnetic beads. (A) Capture chamber in which target proteins are captured on-line from the sample by heavily labeled enzyme-antibody-magnetic beads to form protein-bead bioconjugates. These are washed in the chamber while held magnetically, then transported into the detection chamber (B) in the modular microfluidic system (C). The magnet (D) traps bioconjugate beads in the channel during injection of sample and washing, and is removed for transfer of beads to the detection chamber. Changes in the system for ECL detection include the replacement of multi-electrode array with a solid 2.5x2.5 cm pyrolytic graphite chip with an array of microwells featuring carbon nanotube forests and replacement of the detection chamber with a similar chamber that features a optical window on the top to facilitate detecting ECL light with a CCD camera.

These arrays are fitted into the microfluidic PDMS detection channel, which is attached to a syringe pump or micropumps, with associated valves to deliver reagents and samples to the correct chambers. Magnetic control is used to hold magnetic beads bioconjugated with antibodies and enzyme labels in the capture chamber.

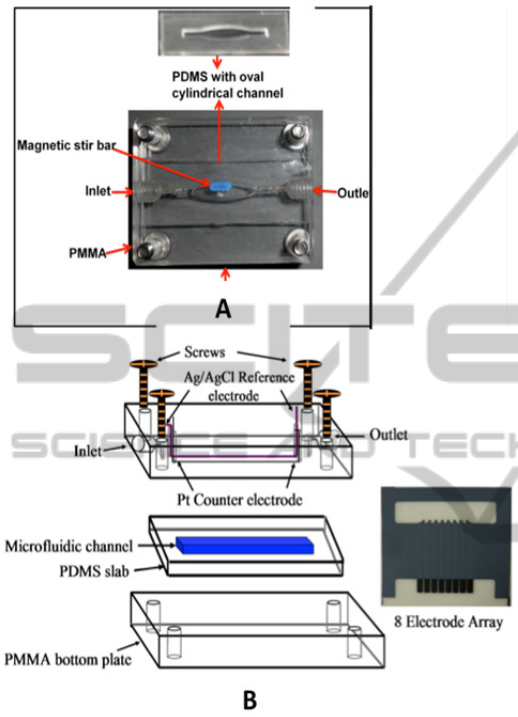


Figure 2: Modules of amperometric microfluidic arrays with on-line protein capture on magnetic beads. (A) Capture chamber featuring a PDMS-defined 100 µL oval cylinder sandwiched between two hard PMMA plates. A tiny magnetic stir bar is included for mixing and redispersing beads. The target proteins are captured on-line from the sample in this chamber by heavily labeled enzyme-antibody-magnetic beads to form protein-bead bioconjugates. These are washed, and then transported into (B) detection chamber housing an 8 AuNP-coated electrode sensor array (on right, sensor elements on top, contacts on bottom) coated with capture antibodies.

Subsequently, samples are injected and flow times adjusted so as to fill the sample chamber. Washes are done to minimize non-specific binding, and then sent to waste. Then, magnetic control is released, and the beads are transported to the detection chamber, where a stop-flow incubation period allows capture by antibodies on the sensors. After washing, an enzyme activator/mediator solution is injected to provide low noise, peak-shaped responses, as shown for a mixture of interleukin (IL) proteins IL-6 and IL-8 in serum (Figure 3).

Our multilabel approaches feature massively enzyme- or ECL-dye-labeled particles that greatly amplify signals for analyte proteins. Detection limits as low as 5 fg $mL^{-1}$ (~200 aM) have been achieved for simultaneous measurement of four oral cancer biomarker proteins in a few µL of serum in about 1 hr (Malhotra, et al., 2012). Applications to biomarker proteins for oral cancer, metastasis, and inflammation have been pursued. For detection of metastasized cancer during surgery, high sensitivity can be traded for speed to achieve immunoassays in 8 min (Krause, et al., 2013). The modular system can be adapted to other measurements such as oxidized DNA and metabolic toxicity screening by changing the active films on the detectors.

## 3 AUTOMATED REAGENT AND SAMPLE INTRODUCTION

POC protein detection will require adding further automation to the above prototypes. We have thus most recently designed an automated reagent and sample delivery module for ECL detection of proteins. The system features six microfluidic channels that lead to a detection chamber featuring a patterned pyrolytic graphite-SWCNT chip (Figure 4). Detection is facilitated by an ECL dye embedded into 100 nm silica nanoparticles and coated with antibodies to provide amplification and low fg/mL detection levels using a CCD camera. The entire device costs ~€500, excluding the CCD camera. Microwells were fabricated on the PG chip using the print/heat/peel technology described above. Integrated micropumps, one per channel, were connected to a portable sample/reagent loading cassette with preloaded, serum samples, wash buffers and dye-silica nanoparticles equipped with detection antibodies. These air-separated solutions are pumped into the six-channel measurement chamber chip with SWCNT wells containing capture antibody). A microcontroller open-source electronics prototyping platform (Arduino) interface provides fully automated control of flow and incubation times. A panel of 4 cancer biomarkers can be measured at clinical levels using this approach.

## 4 CONCLUSIONS

We described inexpensive, versatile microfluidic devices for multiple protein detection designed in a

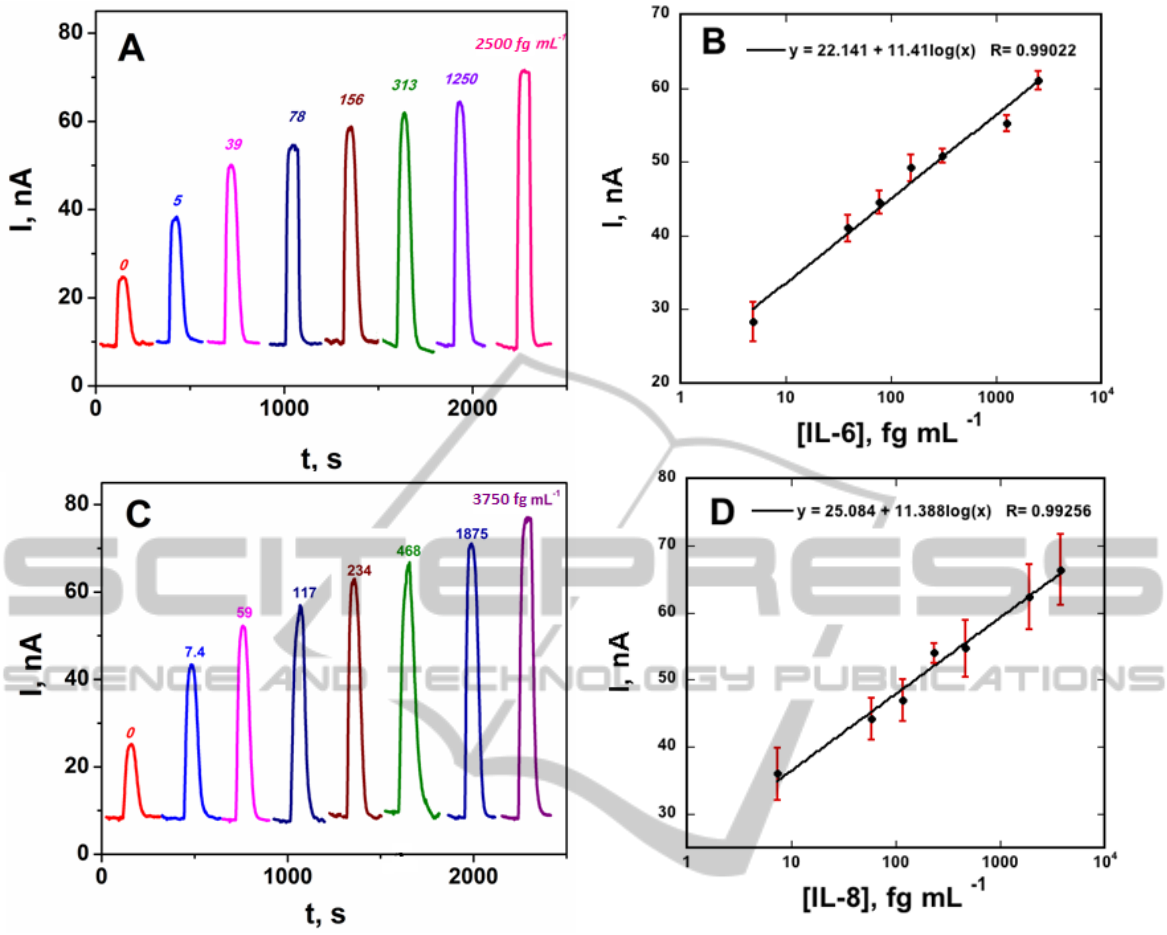


Figure 3: Amperometric immunoarray results for protein mixtures in undiluted calf serum using an 8-electrode screen-printed carbon array with AuNP-coated sensors in the device in Figure 1 at -0.2 V vs. Ag/AgCl. Measurements were done with an 8-electrode CH Instruments potentiostat. Peaks are developed by injecting a mixture of 1 mM HQ and 0.1 mM $H_2O_2$ to mediate and activate the horseradish peroxidase (HRP) labels on the magnetic beads for (A) IL-6 and (C) IL-8, and calibration plots for (B) IL-6 and (D) IL-8. Error bars represent standard deviations (n=4) (Adapted from Otieno et al., 2014).

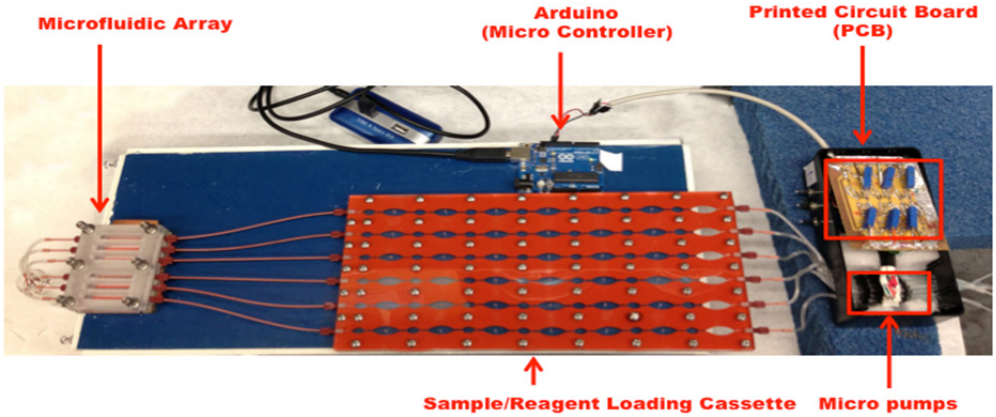


Figure 4: Modular microfluidic array for ECL detection of proteins showing hardware including automated sample and reagent loading cassette. The microfluidic array is placed under a CCD camera in a dark box and connected to a power supply for ECL measurements.

modular fashion with no lithography required, using commercial valves and pumps when possible. Printing, wet etching and print patterning have been used to fabricate very inexpensive nanostructured array chips. These devices can be set up in almost any laboratory at a relatively low cost. However, additional simplicity of operation is a goal for full POC implementation. Our specific application is detection of multiple proteins from serum and cell lysates for cancer diagnostics, and this can be achieved at detection limits down to 5 fg/mL (attomolar levels), up to 200 times lower than existing commercial multiplexed protein detection systems (Rusling J. F., Kumar C. V., Gutkind J. S., et al. 2010).

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