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Integrated optical detection of autonomous capillary microfluidic immunoassays:a hand-held point-of-care prototype

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ABSTRACT

The miniaturization of biosensors using microfluidics has potential in enabling the development of point-of-care devices, with the added advantages of reduced time and cost of analysis with limits-of-detection comparable to those obtained through traditional laboratory techniques. Interfacing microfluidic devices with the external world can be difficult especially in aspects involving fluid handling and the need for simple sample insertion that avoids special equipment or trained personnel. In this work we present a point-of-care prototype system by integrating capillary microfluidics with a microfabricated photodiode array and electronic instrumentation into a hand-held unit. The capillary microfluidic device is capable of autonomous and sequential fluid flow, including control of the average fluid velocity at any given point of the analysis. To demonstrate the functionality of the prototype, a model chemiluminescence ELISA was performed. The performance of the integrated optical detection in the point-of-care prototype is equal to that obtained with traditional bench-top instrumentation. The photodiode signals were acquired, displayed and processed by a simple graphical user interface using a computer connected to the microcontroller through USB. The prototype performed integrated chemiluminescence ELISA detection in about 15 min with a limit-of-detection of ≈ 2 nM with an antibody-antigen affinity constant of $\approx 2 \times 10^7$ M^{-1}.

1. Introduction

Over the last few decades, miniaturized, integrated Lab-on-Chip (LoC)s have been emerging as a technology with potential for development into industrial products. In general, LoCs result from the integration of microfluidics with sensors (Mark et al., 2010). With respect to traditional laboratory fluid handling, microfluidics have advantages such as increased portability, decreased time of analysis due to the decreased diffusion distances and lower cost due to the lower volume of reagents required. Moreover, miniaturization allows the possibility of multiplexing as well as the use of high-throughput methods (Foudeh et al., 2012; Goluch et al., 2009; Miller et al., 2012). These advantages make microfluidics particularly attractive for next-generation systems for cell culture and manipulation (Zervantonakis et al., 2012; Sharei et al., 2013), chemical and bio-chemical analysis (Livak-Dahl et al., 2011; Miller et al., 2012), among other applications, both in the laboratory or at the Point-of-Care (PoC) level.

PoC devices aim at providing fast, on-the-spot diagnostics without requiring the need for a sample to be taken to a central laboratory for analysis. Although PoC is a term traditionally used to describe clinical devices it should be understood in a more generic context as a diagnostic tool that can be used for a wide range of applications including monitoring of food quality (Novo et al., 2013a), and environment and sanitation safety (Deiss et al., 2014). Rapid analysis and ease of operation are required in PoC testing. However, the implementation of microfluidic devices has been limited due to obstacles at the fluid input/output and at the flow control, user interface, and automation levels. The integration of active actuation of solutions (*e.g.* using syringe, acoustic or peristaltic pumping; pressurized or electromechanical valving; connecting tubing; etc.), has a direct impact on the complexity and cost of the systems and for some applications may be a limiting factor in the development of a product. Capillary microfluidic strategies allow for passive fluid flow, avoiding the use of external pumping system, tubing and couplers, while generally allowing for simple and easy introduction of the fluids (Novo et al., 2013b; Safavieh and Juncker, 2013; Chen et al., 2012; Lutz et al., 2011; Gervais and Delamarche, 2009). Sequential, autonomous fluid delivery can be obtained either with microchannels (Novo et al., 2013b; Safavieh and Juncker, 2013) or with paper-based capillary devices (Chen et al., 2012; Lutz et al., 2011). However, the use of closed channels allows for a higher degree of control of the dynamics of the liquids flow as compared to paper devices, especially with regard to passive valving. Furthermore, the use of capillary

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based microchannel devices allows the use of transparent materials such as poly(dimethylsiloxane) (PDMS), poly(methy methacrylate) (PMMA), glass, and OSTE (Saharil et al., 2012), which is important for optical detection methods, and allows more flexibility at the chip design level (*e.g.* if cell culture chambers, chemical and bio-chemical reactors, need to be integrated in the design).

Standard laboratory diagnostic techniques comprise step-sequenced protocols, involving different liquid solutions, that result in a change of a physical property (optical, electric, magnetic, pH) as a function of the concentration of the analyte under test. Suitable bench-top instrumentation (*e.g.* microscopes, electrometers, current amplifiers) and sensors (*e.g.* CCD cameras) are required to monitor and quantify the changes of these properties. The same is true for LoC miniaturized devices. Numerous reports in the literature have successfully demonstrated the integration of miniaturized optical (Novo et al., 2013a), magnetic (Freitas et al., 2012), and electrochemical (Wee et al., 2013; Malhotra et al., 2012) sensors with microfluidic channels. For instance, *E. Wee* and co-workers demonstrated the detection of DNA base changes in breast cancer cell lines by integrating electrodes in microfluidics for measuring electrochemical changes produced by the enzyme horseradish peroxydase (HRP) (Wee et al., 2013). Despite the demonstrated potential of developing LoCs into PoC devices, the number of reports published is still relatively reduced (Yetisen et al., 2013; Chin et al., 2012). As mentioned above, the implementation of LoCs into commercial products, namely for PoC, has been plagued especially by fluid handling and device/user interface obstacles. Simplifying the instrumentation for both the measurement of the sensors' output and the automation of the analysis is also challenging. Nevertheless, there have been increasing reports of PoC prototype development in the literature: *W. Jung* and co-workers have recently demonstrated a chemiluminescence based PoC prototype for the quantification of thyroid stimulating hormone (TSH) with a limit-of-detection (LoD) of 1.9 μIU/ml, useful of the diagnostic of hypothyroidism which, according to that work, occurs for TSH concentrations above 10 μIU/ml (Jung et al., 2013); *M. A. M. Azmi* and co-workers showed the detection of the prostate cancer risk biomarker 8-hydroxydeoxyguanosine at concentrations as low as 1 ng/ml using silicon nanowire-based biosensors integrated with a hand-held readout device (Azmi et al., 2014). Optical based detection systems have been especially successful, notably with the use of smartphones as reviewed by *H. Zhu* and co-workers (Zhu et al., 2013).

In this paper, we demonstrate a PoC prototype as an integrated, hand-held and user-friendly platform with general applicability. By integrating capillary microfluidics with microfabricated hydrogenated amorphous silicon (a-Si:H) photodiodes as transducers and electronics for data acquisition, we have assembled a device of simple operation accessible to the public in general. First, the development of an optical detection setup comprising microfabricated photodiodes connected to a transimpedance amplifier that converts the sensor's current into voltage with a gain of $\approx$217 dB or 6.8×10^{10} Ω is demonstrated. A Teensy 3.0 microcontroller, integrated with the amplifier board, measures the circuit's output voltage through a 16-bit analog-to-digital converter (ADC). The electronic setup has sub 10 fA LoD and a sensitivity with a linear response over a range of 50 pA. The microfluidic module is a capillary based device capable of autonomous and sequential fluid flow without the need for actuation. Finally, the PoC prototype is validated by performing a model microspot-based chemiluminescence Enzyme Linked Immunosorbent Assay (ELISA) using the capillary microfluidic device with integrated detection. User intervention is only required for placing of the solutions at the microchannels' inlets in the beginning of the analysis and inserting the microfluidic device into the PoC prototype box. The results showed a LoD of $\approx$2 nM and an antibody-antigen affinity constant about of 2×10^7 M^{-1} which are in good quantitative agreement with previous work (Novo et al., 2011, 2013b).

2. Materials and methods

2.1. Photodiode fabrication

Arrays of 100×100 μm^2 a-Si:H photodiodes spaced by 800 μm were microfabricated on Corning display quality glass substrates (either Eagle XG or 1737). The aluminum (Al) bottom contacts were deposited by magnetron sputtering, patterned by photolithography and etched by wet etching (using Gravure Aluminium Etchant from Technic, Microchemicals). The *n-i-p* a-Si:H photodiodes were deposited by plasma-enhanced chemical vapor deposition (PECVD) (13.56 MHz) at a substrate temperature of 250 °C, a power density of 50 mW/cm^2, and a deposition pressure of 0.1 Torr. Doped *n*-type and *p*-type films were obtained by addition of phosphine (5 standard cubic centimeters per minute (sccm) diluted to 2% in hydrogen) and diborane (5 sccm, diluted to 2% in hydrogen) gases to 10 sccm of pure silane during film growth, respectively. The *n-i-p* stack was patterned and etched by reactive ion etching (RIE) using a mixture of SF_6 and CHF_3 gases. Amorphous silicon nitride (a-SiN_x) was deposited by PECVD for insulation of the diodes sidewalls, leaving a via access for electrical contact on the top of the photodiodes opened by lift-off. A transparent top contact made of indium tin oxide (ITO) was deposited by sputtering and patterned by lift-off. The top contacts (*p*-layer) of all the photodiodes were shorted through the ITO contact (common anode configuration). Aluminum lines were deposited and patterned, as described above, for completion of the top contacts. A second a-SiN_x passivation layer, 300 nm thick, was deposited by PECVD for protection of the microfabricated structures. Access to pads was made by RIE etching.

The photodiode chips (12×7.5 mm^2) were diced and wirebonded to custom designed printed circuit boards (PCB). Fig. 1-B shows an image of the PCB after milling to create a two level pocket on the left side of the PCBs: one deeper (1 mm deep – blue highlighted area), central, to accommodate the photodiode chip (0.7 mm thick) and another shallower (0.25 mm deep – red highlighted areas) on the sides, for aligning of the microfluidic device with the photodiode chip. Thus, mechanical contact between the photodiode chip and microchannel device is avoided while maintaining a minimal distance of $\approx$50 μm between the detector and the microfluidic channel where the reaction takes place. The chips were mounted on the designated pockets and wirebonded to the PCB. Fig. 1-C shows a magnified perspective view of the photodiode chip area.

2.2. Photodiode current amplifier circuit and hand-held prototype unit

All integrated circuits used are from Texas Instruments. The amplifier circuit, represented in Fig. 2-A, comprises two amplification stages. The first is a transimpedance amplifier with a gain of 180 dB set by a 1 GΩ resistor with a negative feedback to a LMC660 op-amp. The second is a difference amplifier with a 40 dB gain using an OPA4344 op-amp. A stable 2.5 V output, generated by a circuit implementing a REF5025 low-noise, very low-drift, precision voltage reference and an OPA350 op-amp, is used as a virtual ground (VGND) in the transimpedance amplifier. The photodiode anode is connected to VGND (which in turn is connected to the LMC660 non-inverting input) while the cathode is connected to the amplifier's inverting input (therefore placing the photodiode at zero bias). The difference amplifier subtracts VGND to the transimpedance stage output and is followed by a voltage divider which confines the whole amplifier circuit output within the ADC voltage input range (0–3.3 V). Thus the resulting amplification gain of the photodiode signal is of $\approx$217 dB.

The circuit was soldered to a double sided PCB as shown in Fig. 2-B. A Samtec edge card socket (part # MB1-120-01-F-S-02-SL) soldered to the bottom of the board is used to connect the circuit