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Microfluidic Point-of-Care Diagnostics for Resource-Poor Environments

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Abstract – **Point-of-care (POC) diagnostics have tremendous potential to improve human health in remote and resource-poor settings. However, the design criteria for diagnostic tests appropriate in settings with limited infrastructure are unique and challenging. Here we present a custom optical reader which quantifies silver absorbance from heterogeneous immunoassays. The reader is simple, low-cost and suited for POC diagnostics.**

I. INTRODUCTION

THERE is tremendous interest in developing microfluidic systems for applications since they offer many potential advantages including reduced reagent volume, low cost, short analysis times and small size [1-4]. Because of these qualities, microfluidics is an attractive technology for point-of-care applications in resource-poor environments. Improved and appropriate point-of-care diagnostics have potential roles in health care such as providing suitable and prompt treatments, ensuring safe blood banking and improving clinical outcome [2, 6]. Lack of access to diagnostic tests in developing countries often leads to treatments based on clinical symptoms and predominance of disease, which may not be needed or appropriate and contribute to drug resistance [6]. However, to operate in such challenging environments, a microfluidic device need to be designed such that it can operate under constraints of untrained workers, electricity shortage, limited laboratory supplies, and rough handling during transportation and storage [2, 5].

Immunoassays are an important class of diagnostics in global health. The most common format is enzyme-linked immunosorbent assay (ELISA), which are primarily found in centralized laboratories (with significant infrastructure). ELISA is a heterogeneous immunoassay in which analytes from a sample solution bind to capture proteins immobilized on solid substrates [3, 7]. The method relies on secondary antibodies conjugated with enzymes to amplify colorimetric or luminescent signal. However, ELISA typically requires expensive and bulky optical detection instruments which make the use of ELISA in resource-poor environments difficult [5].

One attractive solution which offers a visual macroscopic readout but is compatible to microfluidics is silver reduction amplified by gold-nanoparticles [5]. Silver reduction is compatible with continuous flow, and can be detected and quantified using low-cost optics, unlike other detection methods (such as fluorescence), which typically require expensive and complicated instruments [5, 8]. Using simple optical detection, gold nanoparticle-conjugated secondary antibodies are used instead of enzyme-conjugated antibodies; these metal particles catalyze reduction of silver ions to silver atoms in the presence of a reducing agent (*e.g.* hydroquinone). The resulting silver film attached on transparent plate has an opacity (partially blocks the transmission light) which is directly correlated to the concentration of the analyte [5].

We present an inexpensive custom-built reader to detect the silver signal over the microchannels and display the light intensities. This reader is suited to resource-poor environments as it is low-cost, sensitive, compact, and consumes little energy [2].

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II. METHODS

A. Reader design

The custom-built reader is designed for analyzing the optical signals from microfluidic chips. The reader consists of a detection unit and a display unit, with all components controlled by an Atmel Mega32 microcontroller. The detection unit consists of a super bright LED (660 nm) as a light source and a photodetector (photo sensitivity at 660nm is 0.36 A/W) (*Hammamatsu*) to measure the light passing through the silver film on microfluidic chips. The intensity of transmitted light is converted from analog signals to digital values by an A/D converter and displayed on a liquid crystal display (LCD) (*Digikey*). They are used to calculate the absorbance using equation (1). To reduce noise from the surrounding light, black Delrin plastic plates are used to cover the microfluidic chips and hold the electronic and optic components in place. Furthermore, a 2 mm diameter pinhole is aligned on top of the photodetector to eliminate stray light that is outside the detection zone. The 5.5 x 5.5 x 4.5 inches device is operated by a 9 V battery (or an AC adaptor), which is suitable for use in resource-poor environments (without ground electricity).

B. Power analysis

We summed the power consumption of each component to estimate total power consumption of the reader. For each test, LED, microcontroller and LCD consumes 10.5, 60, and 10 mW respectively. Since the device runs no more than 30 seconds per test, we

calculate that 2.4 J is consumed for each position. By using a 9 V battery, the reader can be operated more than 8,400 times, or 70 hours continuously.

C. Fabrication of microfluidic chips and immunoassay procedures for reader characterization

We characterized the efficiency of the reader by performing an immunoassay using silver reduction in polydimethylsiloxane (PDMS) chips [5] to detect human IgG antibody. In order to prepare the microfluidic immunoassay, human IgG (*Sigma-Aldrich*) 10 µg/mL in phosphate buffered saline (PBS) is spotted on plastic substrates and incubated in humid chamber for 2 hours at room temperature. After that, we washed each spot three times with PBS, rinsed with deionized water and dried with nitrogen gas. Then, we sealed the PDMS chip on the plastic substrate and blocked with 3% BSA–0.2% Tween-20 (in PBS) (*Sigma-Aldrich*) to prevent non-specific protein adhesion to channel surfaces. To perform the assay, we loaded gold conjugated anti human IgG antibody in the channel, followed by two plugs of PBS-Tween 20 and four plugs of deionized water. Next, we loaded the silver solution (a mixture of silver salts and reducing agent; *Sigma-Aldrich*) to the channel and flowed for 3 minutes. Figure 1 provides the immunoassay procedure to detect human IgG. The silver film intensities were measured by the reader and calculated to absorbance values. The absorbance (optical density) can be determined from [9]

$$A_\lambda = -\log_{10}\left(\frac{I}{I_0}\right), \qquad (1)$$

where I is the intensity of light at a specified wavelength λ that passes through a silver opaque and I_0 is the intensity of the light that passes through a blank channel.

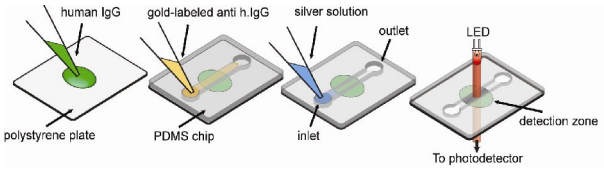

Fig 1. The flow chart briefly shows the immunoassay procedure to detect human IgG.

III. RESULTS AND DISCUSSION

To detect the optical density of silver development over a small region, we used a simple and low-cost reader containing components available from commercial vendors for $55. Figure 2 shows the schematic diagram of the reader. Combining this reader with microfluidic-based immunoassays is beneficial for POC diagnostics under real-world conditions.

We demonstrated the detection of human IgG antibody using a microfluidics-based immunoassay with silver reduction. The silver film opacities of human IgG were measured by the reader and converted to absorbance values. Figure 3A shows the absorbance values from an immunoassay detecting different concentrations of captured human IgG. The optical densities calculated from the reading values are proportional to the concentrations of captured human IgG (and more directly to bound secondary gold-conjugated anti-human IgG antibodies), as silver film opacity is a function of the concentration of the analyte.

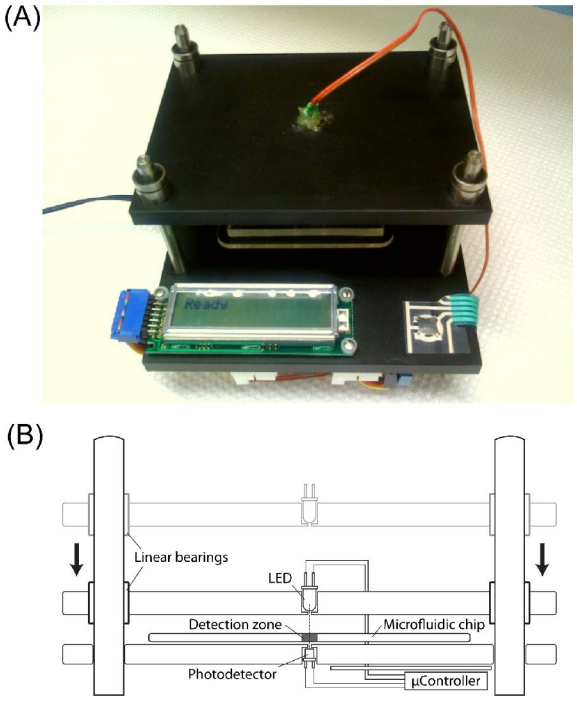

Fig 2. Reader schematic. (A) A photo of the custom-build reader showing the user interface and detection unit. (B) Schematic of the instrument components showing optics, electronics and component structure.

Figure 3B represents the reproducibility of the reader. The coefficient of variance (CV) (calculated from five optical densities of each analyte zone) shows that the higher ODs tend to have lower CVs (with most ranging from 0 – 0.2), and that lower ODs tend to have higher CVs (with most ranging from 0 – 0.4). However, in the low OD regime, the standard deviation is low (< 0.01), which allows resolution of fine greyscale levels. The resolution and range of this reader makes it suitable for semi-quantitative analysis in POC diagnostics.

One issue in signal detection is that the small region of microchannels limits the sensitivity of assays using simple optical detection [5]. Therefore, the sensitivity of the reader needs to be investigated in order to complement the ability of microfluidic systems to detect small quantity of analytes. Further work will address the detection limit of the reader.

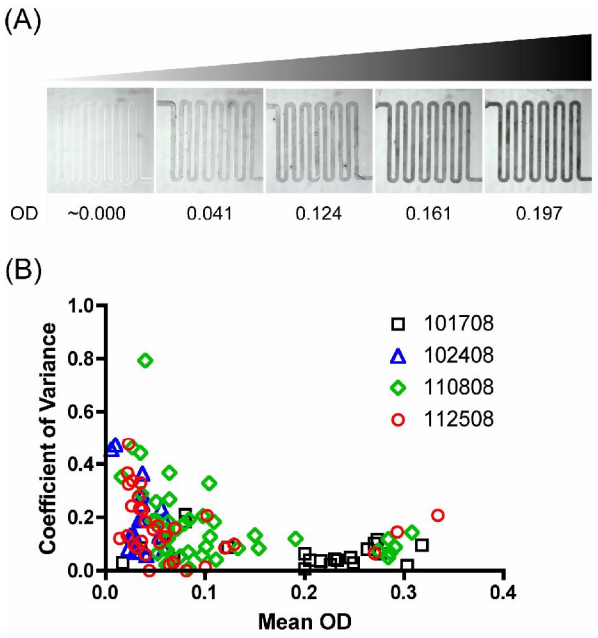

Fig 3. Reader characterization. (A) The gradients of optical density. (B) The coefficient of variance of each mean optical density.

IV. CONCLUSION

The custom-built reader for resource-poor environments is created under constraints of cost, low power consumption, ease of use, rapid analysis, and portability. The silver reduction method is compatible with the use of microfluidics [5] and simplifies detection procedure by measuring absorbance values. The reader may be applied to detect absorbance signals from any microfluidic platforms intended for point-of-care diagnostics in global health.

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