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Thousand-fold fluorescent signal amplification for mHealth diagnostics

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ABSTRACT

The low sensitivity of Mobile Health (mHealth) optical detectors, such as those found on mobile phones, is a limiting factor for many mHealth clinical applications. To improve sensitivity, we have combined two approaches for optical signal amplification: (1) a computational approach based on an image stacking algorithm to decrease the image noise and enhance weak signals, and (2) an optical signal amplifier utilizing a capillary tube array. These approaches were used in a detection system which includes multi-wavelength LEDs capable of exciting many fluorophores in multiple wavelengths, a mobile phone or a webcam as a detector, and capillary tube array configured with 36 capillary tubes for signal enhancement.

The capillary array enables a ~100 × increase in signal sensitivity for fluorescein, reducing the limit of detection (LOD) for mobile phones and webcams from 1000 nM to 10 nM. Computational image stacking enables another ~10 × increase in signal sensitivity, further reducing the LOD for webcam from 10 nM to 1 nM.

To demonstrate the feasibility of the device for the detection of disease-related biomarkers, adenovirus DNA labeled with SYBR green or fluorescein was analyzed by both our capillary array and a commercial plate reader. The LOD for the capillary array was 5 ug/mL, and that of the plate reader was 1 ug/mL. Similar results were obtained using DNA stained with fluorescein.

The combination of the two signal amplification approaches enables a ~1000 × increase in LOD for the webcam platform. This brings it into the range of a conventional plate reader while using a smaller sample volume (10 ul) than the plate reader requires (100 ul). This suggests that such a device could be suitable for biosensing applications where up to 10 fold smaller sample sizes are needed.

The simple optical configuration for mHealth described in this paper employing the combined capillary and image processing signal amplification is capable of measuring weak fluorescent signals without the need of dedicated laboratories. It has the potential to be used to increase sensitivity of other optically based mHealth technologies, and may increase mHealth's clinical utility, especially for telemedicine and for resource-poor settings and global health applications.

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1. Introduction

Mobile computing, medical sensors, and communications technologies for healthcare (mHealth) (Istepanian et al., 2004) have the potential to address the needs of medical diagnostics and provide clinical utility, especially for applications in telemedicine, in resource poor areas, and in global health settings. It is envisioned that point-of-care diagnostics will improve the practice of medicine and public health by providing better medical access to meet the needs of underserved populations in remote locations where current techniques designed for conventional medical settings are not viable, affordable, or compatible with the conditions of underserved populations (Hay Burgess et al., 2006; Urdea et al., 2006; Yager et al., 2008).

Recently, optical detection technologies based on mobile devices have been developed for mHealth, including a reader for lateral flow immuno-chromatographic assays (Mudanyali et al., 2012), wide-field fluorescent microscopy (Zhu et al., 2011b), capillary array for immunodetection for *Escherichia coli* (Zhu et al., 2012), lensfree microscopy (Tseng et al., 2010), fluorescent imaging cytometry (Zhu et al., 2011a), microchip ELISA-based detection of ovarian cancer HE4 biomarker in urine (Wang et al., 2011), detection systems for melanoma or skin lesion (Boyce et al., 2011; Rosado et al., 2012; Wadhawan et al., 2011), loop-mediated isothermal amplification (LAMP) genetic testing device (Stedtfeld

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et al., 2012), acoustic wave enhanced immunoassay (Bourquin et al., 2011), a colorimetric reader (Lee et al., 2011b), phone-assisted microarray reader for mutation detection (Zhang et al., 2011), and mobile phone cameras for DNA detection (Lee et al., 2011a). Many of these technologies rely on the inherent sensitivity of the CMOS camera native to the mobile devices. However, the sensitivity of these CMOS-based cameras is too low to be useful for many optical modalities, such as low fluorescent signal detection, and none of the aforementioned technologies enhances the sensitivity of the mobile phone camera, which is the limiting factor for optical detection in mHealth applications.

To utilize low cost detectors with high noise levels (e.g., $10 webcams) for mHealth applications, a computational approach known as "image stacking" has been employed recently to improve sensitivity for fluorescent detection (Balsam et al., 2012a). In webcam images, it is difficult to distinguish between the signal and the noise due to the poor signal-to-noise ratio (SNR). However, using the webcam in video mode makes it possible to capture many individual frames and combine them with an image stacking algorithm to average the values of each pixel, so the random noise which is present can be significantly reduced. The resulting stacked image has a significantly higher SNR (and consequently an improved LOD), and consists largely of the underlying signal, which enables the detection of weak signals that would normally be masked by the noise without image stacking.

In addition to computational image enhancement through image stacking, optical amplification of signals can also increase sensitivity. Previously, capillary tubes have been used in medical diagnostic for both assay fluid handling and waveguide illumination (Weigl et al., 1994). Several types of capillaries, optical path geometries, and configurations have been investigated including: (1) vertical excitation (to the long axis) at 90° angle and detection of emitted light (Cosford and Kuhr, 1996), (2) vertical excitation detected at one end (Misiakos and Kakabakos, 1998; Ligler et al., 2002), and (3) horizontal (or angular) excitation through the end of the capillary (Flanagan and Sloper, 1992). In the latter configuration, excitation light propagates through the capillary (or planar) walls to enable evanescent excitation as well as propagation of the emitted light along the capillary with a grating to couple the light out of the waveguide to a detector, and has also been used for evanescent fiber optic biosensors (Anderson et al., 2000). Horizontal excitation generates evanescent wave illumination, which combined with vertical detection has been used mainly for planar (not capillary) detection (Wadkins et al., 1998; Plowman et al., 1999; Rowe et al., 1999; Silzel et al., 1998).

These previously investigated capillary configurations are not suitable for mHealth because they utilize costly components, such as laser illumination, photomultiplier tubes (PMTs), or actively-cooled CCD detectors to improve sensitivity. All of these devices also utilize complex optics configuration, and most have limited portability. This renders them less effective for mHealth applications where the need is for simple, low cost and portable devices. In previous work, a capillary array has been used to perform immunoassay for detection with a mobile phone (Zhu et al., 2012). However, the capillary array was not used for optical signal amplification of the assay, and a high level of detection sensitivity (5–0 cfu mL^{-1} for *E. coli*) was obtained through the use of quantum dots.

In an alternative capillary waveguide design we have developed, the capillaries can be illuminated by a multi-wave length LED light source with light propagating parallel to the capillary axis. The light-wave energy that couples with the capillary propagates through its walls and interacts directly with fluorescent molecules to excite them via evanescent waves, and fluorescent emission can be detected at the end of the capillary by a mobile phone or CCD camera. To increase the sensitivity of mHealth technologies, we combine this capillary design with the computational enhancement via image stacking to increase sensitivity for fluorescence detection. This combined approach has the potential to form the basis to use mHealth technologies for optical detection in assays requiring high sensitivity using low cost medical diagnostic techniques with clinical utility in resource-poor settings for global health.

2. Materials and methods

2.1. Materials and reagents

The 36-channel capillary arrays used for analysis were fabricated using heparinized glass capillaries (Drummond Scientific, Broomall, PA) held in a square array by black poly(methylmethacrylate) (PMMA), also known as acrylic (Piedmont Plastic, Inc. Beltsville, MD). 36-well plates were fabricated using black acrylic sheet to define the well volume and clear polycarbonate sheet to define the well bottom. For bonding the black acrylic with the polycarbonate, 3 M 9770 adhesive transfer double sided tape was used (Piedmond Plastics Inc., Beltsville, MD). To block the waveguiding properties of the glass capillaries some were coated with high purity silver using a sputter deposition chamber (Denton Desk IV, Denton Vacuum, LLC). The fluorescence measurements were made using fluorescein (Sigma-Aldrich Co. LLC) diluted in water as a standard.

2.2. mHealth fluorescence detector

The main components of mHealth fluorescence detector are: (1) an LED excitation source described in previous work (Sun et al., 2010a) capable of producing light from 450 to 650 nm (red 610–650 nm, green 510–550 nm, and blue 450–500 nm). (2) Excitation and emission filters, which for fluorescein is a 20 nm bandpass filter with a 486 nm center wavelength (D486/20 ×) for excitation and a 50 nm bandpass filter with a center wavelength of 535 nm (HQ535/50 M filters, both from Chroma Technology Corp., Rockingham, VT) for emission. (3) An optical detector, which for this work two different types were investigated: (a) generic webcam color video camera (AVEO Corp.) with an 8 bit 640 × 480 pixel CMOS sensor enabling 256 levels of gray scale (typical Ebay vendor) used in previous work (Balsam et al., 2012b; Balsam et al., 2011), and (b) Samsung Galaxy SII smart phone (Samsung Electronics Co.) with a built-in lens with a focal ratio of f/2.65 and a 4 mm focal length. The phone was used with an aftermarket application which allows control of the exposure time and gain of the camera (Camera FV-5, Flavio González Vázquez).

2.3. Fabrication of 36-channel fluidics

***Capillary array fluidics*:** A 6 × 6 array of heparinized glass capillaries was fabricated. To orient all 36 capillary channels toward the camera image sensor simultaneously, two laser machined six-by-six arrays of holes in two 3.2 mm thick plates of black acrylic which hold the capillaries in parallel configuration were fabricated.

***Plate array fluidics*:** The 36-well type plate array was fabricated as described in our previous work (Sapsford et al., 2009; Sun et al., 2009; Sun et al., 2010b). Black acrylic plates with one side coated by 3 M 9770 black adhesive transfer tape were laser machined to have a six-by-six array of wells. A layer of thin polycarbonate sheet was attached to the adhesive transfer tape to form the bottom of the sample wells.

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