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Capillary Array Waveguide Amplified Fluorescence Detector for mHealth

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Abstract

Mobile Health (mHealth) analytical technologies are potentially useful for carrying out modern medical diagnostics in resource-poor settings. Effective mHealth devices for underserved populations need to be simple, low cost, and portable. Although cell phone cameras have been used for biodetection, their sensitivity is a limiting factor because currently it is too low to be effective for many mHealth applications, which depend on detection of weak fluorescent signals.

To improve the sensitivity of portable phones, a capillary tube array was developed to amplify fluorescence signals using their waveguide properties. An array configured with 36 capillary tubes was demonstrated to have a ~100X increase in sensitivity, lowering the limit of detection (LOD) of mobile phones from 1000 nM to 10 nM for fluorescein. To confirm that the amplification was due to waveguide behavior, we coated the external surfaces of the capillaries with silver. The silver coating interfered with the waveguide behavior and diminished the fluorescence signal, thereby proving that the waveguide behavior was the main mechanism for enhancing optical sensitivity.

The optical configuration described here is novel in several ways. First, the use of capillaries waveguide properties to improve detection of weak fluorescence signal is new. Second we describe here a three dimensional illumination system, while conventional angular laser waveguide illumination is spot (or line), which is functionally one-dimensional illumination, can illuminate only a single capillary or a single column (when a line generator is used) of capillaries and thus inherently limits the multiplexing capability of detection. The planar illumination demonstrated in this work enables illumination of a two dimensional capillary array (e.g. x columns and y rows of capillaries). In addition, the waveguide light propagation *via* the capillary wall provides a third dimension for illumination along the axis of the capillaries. Such an array can potentially be used for sensitive analysis of multiple fluorescent detection assays simultaneously.

The simple phone based capillary array approach presented in this paper is capable of amplifying weak fluorescent signals thereby improving the sensitivity of optical detectors based on mobile phones. This may allow sensitive biological assays to be measured with low sensitivity detectors and may make mHealth practical for many diagnostics applications, especially in resource-poor and global health settings.

Keywords

microfabrication; microfluidics; CMOS; fluorescence; mobile phone; smartphone

1. Introduction

Mobile health (mHealth), which is defined as “mobile computing, medical sensor, and communications technologies for healthcare”¹ may enable the practice of medicine and public health using mobile devices. With this enhanced mobility, mHealth has the potential to provide access to medical diagnostics for underserved populations and in remote locations. Currently, most of the research in mHealth has been focused on exploiting the connectivity capabilities of mobile phones²⁻⁶ and not on enhancing the analytical capabilities of mobile devices. At the same time, most analytical diagnostic technologies used today have been developed for laboratory settings in high-income countries, and in many cases are not affordable or compatible with the needs and conditions found in low and middle-income countries. The challenges and the need to develop simple, low-cost diagnostics for resource-poor settings with minimal medical infrastructure are well recognized⁷⁻⁹ and mHealth analytical technologies can be potentially used to overcome the limitations of current medical diagnostics technologies by providing simple and affordable analytical diagnostic technologies that are compatible with mobile phones technologies.

Several detection technologies based on mobile phones have been developed for biodetection, including an integrated rapid-diagnostic-test reader platform for lateral flow immunochromatographic assays¹⁰, capillary array based immunodetection for *Escherichia coli*¹¹, wide-field fluorescent microscopy¹², fluorescent imaging cytometry¹³, lensfree microscopy¹⁴, detection systems for melanoma or skin lesion¹⁵⁻¹⁷, loop-mediated isothermal amplification (LAMP) genetic testing device¹⁸, microchip ELISA-based detection of ovarian cancer HE4 biomarker in urine¹⁹, surface acoustic wave enhanced immunoassay²⁰, a pocket-sized colorimetric reader²¹, phone-assisted microarray decoding platform for signal-enhanced mutation detection²², and mobile phone cameras for DNA detection²³. However, all these technologies rely on the inherent sensitivity of the CMOS camera native to the mobile phone, which is less sensitive than detectors used for biodetection. The camera sensitivity, which is a limiting factor for detection, may be improved through additional hardware or image processing algorithms to improve the performance of these devices.

To improve sensitivity of low cost but high noise detectors (e.g., \$10 webcams) for mHealth applications, a computational approach was developed based on an image stacking algorithm to remove the noise and enhance weak signals for fluorescent detection to improve sensitivity²⁴. While the computational approach increases the sensitivity of fluorescent detection, our unpublished data suggests that this approach may not be suitable for many portable phones because the native camera hardware filters out weak signals in an effort to reduce image noise, which renders computational approaches for weak signal enhancement useless. Since the computational improvement of low signals to increase detection sensitivity was not practical, optical amplification of the signals is needed to increase the level of light intensity from a fluorescent sample to one that is high enough that the phone camera hardware will not reject it as noise.

Improved limit of detection can be achieved using capillaries, which enable both assay fluid handling and waveguide illumination²⁵. While a capillary array has been used previously to performing immunoassay for detection with a mobile phone¹¹, the capillary array was not

used for optical signal amplification of the assay. Instead, a high level of detection sensitivity (5 to 10 cfu mL⁻¹ for *E. coli*) was obtained using quantum dots.

Capillaries have been used in several types of optical biosensors utilizing various excitation and emission modalities, optical path geometries and configurations shown in figure 1 (adapted from reference ²⁶) including: (1) vertical (to the long axis) at 90° angle spot excitation and detection of emitted light ²⁷ (figure 1-1), (2) vertical excitation (e.g. the entire length of the capillary) detected at one end ^{26,28} (figure 1-2), (3) horizontal (or angular) excitation through the end of the capillary with the excitation light propagation through the capillary (or planar) walls enabling evanescent excitation and the propagation of the emitted light along the capillary, utilizing grating to couple the light out of the waveguide to the detector ²⁹, such configuration is also used by evanescent fiber optic biosensors ³⁰ (figure 1-3). Horizontal excitation (in most cases with an angle) generating evanescent wave illumination and vertical detection is used mainly for planar (not capillary) detection ³¹⁻³⁴ (figure 1-4).

However, most published capillary configurations described above are not suitable for mHealth because they require costly components such as laser illumination, photomultiplier tubes (PMTs) or cooled CCD detectors. These devices utilize complex optical configurations, have limited portability or are high cost, whereas effective mHealth devices should be simple, low cost and portable.

To enable the use of portable phones in mHealth applications involving highly sensitive fluorescent detection, we present an alternative optical enhancement approach which uses a standard portable phone with a CMOS imaging sensor combined with LED excitation in a very simple optical configuration: in-line horizontal excitation and horizontal detection. This optical configuration has been used effectively in previous work ^{24,35-42}, however such configuration was used in planar (e.g., microtiter plate) mode and did not take advantage of the waveguide properties of capillaries for signal amplification.

In this work, we developed a new capillary waveguide optical configuration for multiplexed fluorescence detection. While the conventional utilization of evanescent waves for fluorescence detection requires coherent light in the form of lasers ^{26-28,43-52} as the excitation source illuminated in a critical angle and a grating to couple the light out of the waveguide and into a detector ²⁹, in the simple configuration described here the capillaries were illuminated by the multi-wave length LED light emitted horizontally to the capillary axis (figure 1-5). The light-wave energy propagating through the capillary walls can interact directly with and excite the fluorescent molecules *via* evanescent waves. The fluorophores emit light that is detected at the end of the capillary to provide higher detection sensitivity when compared to the same volume of sample being detected in a standard microtiter plate format. This is the basis for using the term *amplification* in this work: the measured fluorescent signal is increased substantially through the use of evanescent wave excitation. This simple capillary array platform is capable of measuring multiple fluorescent-detected assays simultaneously without the need of dedicated laboratories and complex equipment. The approach described here has the potential to form the basis for high sensitivity, low cost medical diagnostics in resource-poor settings for mHealth.

2. Experimental

2.1 Materials and reagents

The 36-channel capillary arrays used for analysis were fabricated using glass capillaries (Drummond Scientific, Broomall, PA) held in a square array by black poly(methyl-methacrylate) (PMMA), also known as acrylic (Piedmont Plastic, Inc. Beltsville, MD). For

bonding the black acrylic with the polycarbonate, 3M 9770 adhesive transfer double sided tape was used (Piedmont 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 Optical components

The main components of mHealth fluorescence detector are: (1) LED excitation source described in previous work³⁸ capable of producing light from 450–650 nm (red 610–650 nm, green 510–550 nm, and blue 450–495 nm). (2) Excitation and emission filters, for fluorescein a 20 nm bandpass filter with 486 nm center wavelength was used (D486/20X), and for emission a 50 nm bandpass filter with center wavelength of 535 nm (HQ535/50M filters, both from Chroma Technology Corp., Rockingham, VT). (3) Two types of detectors were used in this work, a Meade astronomical 16-bit uncooled CCD equipped with a Tamron manual zoom CCTV 4–12 mm, f/1.2 lens (Spartan, Utopia, NY) used in previous work^{36,53} and Samsung Galaxy SII smartphone (Samsung Electronics Co.) with a built-in lens with a focal ratio of f/2.65 and a 4mm focal length. The phone was used with an aftermarket application (Camera FV-5, Flavio González Vázquez). A light-tight enclosure is used to block out all ambient light during to increase sensitivity.

2.3 Fabrication of 36-channel capillary array and 36-well plate array

Two separate fluidic arrays were fabricated: (a) the new capillary array with 36-channels that is the focus of this work, and (b) as a control a plate array with 36-wells for a sensitivity comparison.

Capillary array—An array of 6×6 borosilicate glass capillaries were fabricated with all 36 capillary channels oriented towards the camera image sensor simultaneously. Two laser machined six-by-six array of holes in two 3.2 mm thick plates of black acrylic were fabricated to hold the capillaries in a parallel configuration. The length of the capillaries used is 32mm capillaries, the outer diameter of the capillaries is 0.8 mm, the inner diameter is 0.65 mm with glass thickness of around 50 microns. The capillaries are separated by a distance of 1 mm between their outer walls.

Plate array—The 36-well type plate array fabricated as described in our previous work^{37,39,40} using 3.2 mm thick black acrylic plates with one side coated by 3M 9770 black adhesive transfer tape and 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.

2.4 Digital image signal analysis

Images captured by the detectors were transferred to a personal computer running Windows XP (Microsoft, Redmond, WA). Pixel brightness values were measured using ImageJ software developed and distributed freely by NIH (<http://rsb.info.nih.gov/ij/download.html>). The data generated was then imported into Microsoft Excel (Microsoft, Redmond, WA) for analysis. Images captured with the phone are in 8-bit color JPEG format. Each color image is essentially three different monochrome images, one representing each color channel (red, green and blue), with each pixel having a possible value from 0–255. Because the signal of interest for fluorescein is in the green spectrum, the green channel alone is analyzed and the red and blue channels are discarded.

To reduce background noise in the final image and improve signal to noise ratio (SNR), a median dark frame was subtracted from each image to be analyzed. A dark frame is an image with identical exposure time and gain settings as the original image, but taken with no light reaching the camera sensor (i.e. with a lens cap in place). When several of these dark frames are averaged together, the resulting image represents the average background noise generated by the camera sensor. This averaging was done in ImageJ using many (>30) dark frames with the median value for each pixel calculated and combined to form the final dark frame. The green channel was extracted from these median dark frame images and subtracted from the green channel of the corresponding original image containing the signal of interest. This process has been described in greater depth in previous work^{24,53}. In the current work, all image processing was done manually by ImageJ, but could be easily automated using an application in the phone to simplify and speed up the analysis.

3. Results and Discussion

3.1 Capillary array fluorescence detectors

The main challenge of using a mobile phone for low light fluorescence detection in mHealth applications is the low sensitivity of the phone camera. Wave guiding capillaries were used in this work for evanescent excitation to increase sensitivity.

Detector configuration—The basic configuration of the fluorescence capillary array detectors as shown schematically in figure 2-1 are: (A) a fluorescence excitation source, (B) excitation filter, (C) sample holder array, (D) emission filter, (E) objective lens, and (F) optical sensor. A photograph of the configuration is shown in figure 2-2 where either a cell phone camera or a CCD camera is used as the optical sensor

Capillary array—To increase the detection sensitivity, a new configuration of capillary array was developed, in this capillary waveguide the capillaries are illuminated by the multi-wavelength LED light emitted horizontally (at 486 nm) to the capillary axis (figure 2-3). To take advantage of the LED planar illumination covering a large surface area, the capillaries were arranged in two dimensional 6×6 capillary arrays (e.g. 6 columns and 6 rows of capillaries) which enables increased multiplexing. A schematic image of the array is shown in figure 2-3 and a photo of a capillary array in figure 2-4. To orient all 36 capillary channels towards the camera image sensor simultaneously, two plates of black acrylic with laser-machined 6×6 arrays of holes held the capillaries in a parallel configuration, as shown at the top and the bottom of figure 2-3. Horizontal excitation through the end of the capillary enables illumination of the 2D capillary arrays, which can be used for high throughput, multi-sample analysis. In contrast, vertical illumination of capillaries (figure 1-1 and 1-2) can illuminate only a single column, and thus limits the number of capillaries in the device (e.g., only a single row of capillaries).

Light detection—We used a Samsung Galaxy SII smartphone and a Meade astronomical 16-bit uncooled CCD from our previous work^{42,43} was used as a reference. The lens used for the reference CCD camera is an f/1.2 Tamron manual 4-12 mm CCTV. The built in lens for the mobile phone (figure 2-2-C) has a focal ratio of f/2.65 and a 4 mm focal length.

One challenge in using the mobile phone camera is the limited control on the light exposure. In the phone's built-in camera application, the exposure time is automatically set based on the image brightness. This is not ideal for scientific measurement applications because it makes quantitative comparison of multiple images difficult. To overcome this problem, an aftermarket application was used which allows the user to control the exposure time of the camera (Camera FV-5, Flavio González Vázquez). The range of control is dictated by the

hardware of the image sensor, and the maximum exposure length is 1/15 s for the Galaxy SII phone.

To demonstrate the capability of this new technique, fluorescein (a common dye used in many biological assays) was used as a model fluorescence media. Results were compared with a benchmark diagnostic system that we developed previously^{24,36}.

3.2 Capillary array amplification of fluorescence signal

To study the effect of the capillary array on cell phone camera fluorescence detection, the signal from the capillary array was compared to the signal from a plate. In both configurations the same volume (8.5 μ L) was used. Both configurations were imaged by the mobile phone and CCD camera.

Serial dilutions of fluorescein in the range of 0-3320 ng/ml (0-10,000nM) were analyzed using a ten-fold serial dilution (0.001 nM, 0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM, etc). Exposure time for the cell phone camera was set to 1/15 s and the camera gain was set to its maximum (800 ISO). Figure 3-1 shows the emission image of six concentrations (column A-F in figure 3-1 and 3-3) of fluorescein loaded via micropipette into the 36 capillary array compare to a 36 well plate array (figure 3-3) in six replicas (rows 1-6) recorded with the cell phone camera.

As shown in figure 3-5 the signal from the 36 well plates is lower than the signal image of the 36 capillaries array. The signal intensities from the images were quantified using ImageJ and the dose response curve was plotted. The Signal-to-Noise Ratio (SNR) was calculated as the value measured divided by the control signal (concentration 0 ng/ml). Figure 3-5 is a plot of the mean signal from each concentration of fluorescein as detected by the cell phone camera in both the capillary array and the plate array which suggest that capillary signals are ~100X higher than the plate signals for the same volume of fluorescein. Variation in the signals was found to be less than 5% for a given concentration.

Borosilicate glass is well known to autofluoresce when exposed to blue light. Therefore, a similar experiment was carried out using an Alexa Flor dye with an excitation peak at 654 nm and an emission peak at 674 nm. In this wavelength range, borosilicate does not strongly autofluorescent. As in the fluorescein case, LOD was improved by approximately 100X. In this case, the LOD was not dictated by the autofluorescence of the glass, but instead by the extinction coefficient between the two filters used.

To compare the level of detection of the phone to the level of detection of a very sensitive CCD astronomy camera, the same concentrations of fluorescein were analyzed by a high sensitivity CCD astronomy camera. A capillary array (figure 4-1) and well plate array (figure 4-3) were measured. It can be seen that the same amplification effect is measured with the CCD camera, with a similar amplification level of ~100X. Statistical analysis shows that the limit of detection (LOD), based on three standard deviations of the mean signal for water, is approximately 10nM for the cell phone camera when using the capillary array and approximately 1,000 nM when using the well-plate array. The CCD camera showed a LOD of 0.1 nM with the capillary array and 10 nM with the well-plate array. So while the astronomical grade CCD camera is 100X more sensitive than the cell phone camera, the capillary array increased the cell phone camera sensitivity 100X to achieve the same LOD as the CCD camera when interrogating a plate array.

3.3 The mechanism of waveguide amplification of fluorescence signal

The proposed mechanism for the signal amplification is three-fold. First, the light-wave energy propagating through the capillary walls can interact directly with and excite the

fluorescein molecules *via* evanescent waves. Second, some of the emitted fluorescent light which is emitted under the right conditions will be guided by the capillary walls and will be more effectively directed at the detector. Third, the higher aspect ratio of the capillaries gives those photons traveling directly through the core of the capillary more opportunities to interact with a fluorophore. This third piece is strictly geometric and could be taken advantage of in a well plate with very deep wells.

To demonstrate the effect of the two waveguide portions of amplification, and compare them to the amplification that would be seen by a high aspect ratio well plate array, two different sets of capillary tubes were used: one set was uncoated glass which could act as a waveguide (figure 5-1), and one set was coated on all of its external surfaces with high-purity silver which interferes with total internal reflection and prevents the capillary from acting as a waveguide (figure 5-2). The array was illuminated by the LED illumination module equipped with a blue excitation filter, and the array was analyzed with a green emission filter as previously described. Both capillaries were loaded with four concentrations of fluorescein (figure 5-3, rows 1-4) each in three replicas (columns A-F) and the fluorescent signal of silver coated capillaries (figure 5-3, columns A, C and E) was compared to the signal of uncoated capillaries capable of waveguide propagation (Figure 5-3 columns B, D and F). The signals of the capillaries were detected by the CCD digital camera equipped with an f/1.2 Tamron manual 4-12 mm CCTV lens and the corresponding ImageJ 3D image is shown in figure 5-4. Very weak signal from the highest fluorescein concentrations (100 nM) was measured in silver coated capillaries A-I, C-I and E-I (marked with circles) in comparison to the signal of capillaries without coating (B-I, D-I and F-I) capable of detection at lower fluorescein concentrations (row #2: 10 nM, row #3: 1 nM, row #4: 0.1 nM). The data suggest that signal can be detected with the uncoated capillaries (columns B, D and F) down to a concentration of 0.1 nM, while no signal was statistically detectable in the coated capillaries below 10 nM (column A, C and F).

This experiment demonstrates that amplification is primarily due to the waveguiding nature of the capillaries, with the most likely cause being evanescent waves providing excitation to the fluorescent solution along the entire length of the capillary. The silver coating prevents waveguiding in two ways: first, by blocking light from being coupled into the ends of the waveguide by the excitation source, and second by frustrating any possible total internal reflection and thus blocking waveguide propagation. It is also demonstrated that the proposed geometric amplification due to the high aspect ratio does not improve LOD by a factor of 10 or more, as the LOD of the non-guiding capillaries and the well plate array were the same.

In order to determine if these results translate to other popular phone platforms, an iPhone 4 was used under the same experimental conditions to measure fluorescent solutions loaded in the capillary array and the plate array with wells. The same LODs (10 nM and 1000 nM, respectively) were measured in this case.

4. Conclusions

Many detection technologies based on mobile phones have been developed for biodetection¹⁰⁻²³. However, the sensitivity of the mobile phone camera is a limiting factor: it is currently too low to be effective for many of the diagnostic applications of these devices. To enable the use of mobile phones for mHealth diagnostics involving highly sensitive optical detection, we developed an optical detection approach which combines capillary waveguides with LED excitation in a very simple optical configuration: inline horizontal excitation and horizontal detection. Our approach described here enables 100X improve sensitivity of imaging based fluorescent detection. We demonstrated that the

primary mechanism for the signal amplification is due to light-wave energy propagating through the capillary walls and interacting directly with the fluorescein molecules. When total internal reflection in the capillaries was frustrated, no amplification was measured.

The use of a capillary array configuration takes advantage of the LED planar illumination covering a large surface area, which enables illumination of the two dimensional capillary arrays (e.g. x columns and y rows of capillaries) while the waveguide light propagation via the capillaries wall provides a third dimension for illumination along the axis of the capillaries. The two dimensional capillary arrays enables an increase in the detector multiplexing. In contrast, conventional angular laser waveguide illumination is spot (or line) which is functionally one-dimensional illumination limited to a single row. In this configuration, light-wave energy propagating through the capillary walls can interact directly and excite fluorescent molecules, which emit light that is guided through the capillary wall to provide an improved detection limit (~100X increase) which enables the detection of weak fluorescent signals.

Effective mHealth analytical devices for underserved populations especially in remote locations should be simple, low cost and portable. The device described here address these constraints using components (except the optical filters) that are low cost, readily available, and in an optical configuration that is simple to fabricate. The capillary array in the device is designed to be reusable, and the mobile phones with cameras used for optical detection are ubiquitous. The approach described here to overcome the limiting factor of the phone camera sensitivity can be incorporated into other mobile phone based detectors described in previous works¹⁰⁻²³ enabling them to improve their sensitivities. This approach has potential for developing highly sensitive, low cost mHealth optical diagnostic platforms for resource-poor and global health settings.

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Author biographies

Joshua M. Balsam studied Mechanical Engineering at the University of Maryland (College Park, MD) and in 2009 received his BS in the field. In 2010 he began research in microfluidic biological sensors with Dr. Avraham Rasooly at the US Food and Drug Administration (FDA) in White Oak, MD. Currently he is an engineering PhD candidate at the University of Maryland under Dr. Hugh A Bruck, as well as a research assistant at the FDA in the Office of Science and Engineering Laboratories (OSEL), Division of Biology (DB). His work involves the development of low cost optical point of care biosensors for global health and low resource settings.

Prof. Hugh A. Bruck received BS and MS in Mechanical Engineering in 1988 and 1989 respectively, and his PhD in Materials Science from Caltech in 1995. After working at Idaho National Engineering Laboratories, he came to the Department of Mechanical of Engineering at the University of Maryland as an Assistant Professor in 1998 and is currently Professor and Associate Chair for Academic Affairs working on functional materials and nanoscale property characterization. His current research has led to the development of a new combinatorial technique for formulating polymer nanocomposites and highly filled polymers, new processing-structure-property models for multifunctional polymer nanocomposites, new label-free biosensors based on electrical percolation and enhanced chemiluminescence in bio-nanocomposites, and new multiscale characterization approaches

for hierarchically-structured polymer composites and biological materials. He has authored or co-authored 9 book chapters, 78 journal papers, and 81 conference papers through support from ONR, AFOSR, NSF, FDA, ARO, ARL, NAVAIR, and NAVSEA. His work during that time has been recognized with many honors and awards, including the Best Paper Award at the 2011 ASME Mechanisms & Robotics Conference, A.J. Durelli Award, ONR Young Investigator Program Award, and Fulbright Scholar Award. He currently serves on the International Advisory Board for the journal *Experimental Mechanics*, and was named a fellow of ASME in 2008.

Dr. Avraham Rasooly joined the Food and Drug Administration (FDA) as a Microbiologist in 1995, where he developed microbial detection approaches for foodborne pathogens and their toxins using biosensors and DNA microarrays. In 2002, Dr. Rasooly joined the National Cancer Institute (NCI). He currently serves as a Special Assistant for Cancer Technologies and Translational Research at the NCI, overseeing research in technology related to cancer, and as a researcher at the FDA's Center for Devices and Radiological Health (CDRH) directing a research lab studying new technologies for rapid bio-detection including nanotechnology, biosensors and lab-on-a-chip.

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Highlights

The combination of the two optical approaches, a new capillary waveguides with LED excitation enables ~100X increase in fluorescence signal sensitivity. This approach can improve the sensitivity of many mHealth detectors and expand mHealth clinical utility for many diagnostics applications.

Figure 1. Capillary optical Configuration

Excitation light can illuminate the capillaries from several angles: a 90° angle (1,2) or angular/horizontal illumination utilizing waveguiding and evanescent illumination (3,4 and 5). The fluorescent signal emission can be detected at a 90° angle from the capillary (1,3) or horizontally after coupling into the waveguide and detected from the end of the capillary (2,4 and 5).

Figure 2. mHealth capillary array fluorescence detectors

(1) A schematic configuration of the mHealth capillary array fluorescence detectors with the main system components highlighted in the schematic: [A] a portable phone or digital camera mounted in a homemade acrylic box, [B] lens [C] emission filter mounted on the end of the lens [D] capillary array, [E] excitation filter and multi-wavelength LED. (2) A photo of a portable phone-based fluorescence detector. A schematic image of capillary array is shown in (3) and a photo of the array is shown in (4).

Figure 3. Capillary Array Fluorescence Amplification

Portable phone image of (1) the 36 capillary array loaded with 6 replicas (columns) of 6 different fluorescein concentrations (rows). (2) The corresponding ImageJ 3D image of (1). (3) Image of the 36 well plate array. (4) The corresponding ImageJ 3D image of (3). (5) Signal intensities from the image were quantified using ImageJ and the dose response curve was plotted on a log-log plot, triangles for the capillary array and circles for the well plate array. The assay units are Signal to Noise Ratio (SNR), with each measured value divided by control (concentration 0 ng/ml). The error bars are equal to one standard deviation ($n=6$). The fluorescein concentrations, from left (column A) to right (column F), are 0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM and 10,000 nM. Each concentration was measured 6 times (rows 1-6). Limit of detection SNR is illustrated by a horizontal dashed line.

Figure 4. CCD camera detection of Waveguide Capillary Array Fluorescence

CCD camera image of (1) the 36 capillary array loaded with 6 replicas (columns) of 6 different fluorescein concentrations (rows). (2) The corresponding ImageJ 3D image of (1). (3) CCD image of the 36 well plate array. (4) The corresponding ImageJ 3D image of (3). (5) Signal intensities from the image were quantified using ImageJ and the dose response curve was plotted, triangles for capillary array signal measurements and the circles for plate array measurements. The assay units are Signal to Noise Ratio (SNR), with each measured value divided by control (concentration 0 ng/ml). The error bars are equal to one standard deviation ($n=6$). The fluorescein concentrations, from left (column A) to right (column F), are 0.01 nM, 0.1 nM, 1 nM, 10 nM, 100 nM and 10,000 nM. Each concentration was measured 6 times (rows 1-6). Limit of detection SNR is illustrated by a horizontal dashed line.

Figure 5. Waveguide Fluorescence Capillary Array Amplification

Schematics of capillary with waveguide propagation through the capillary wall (1) and schematics of capillary with waveguide propagation blocked with silver coating. (2); an image with the actual capillaries (3) showing 6×4 array with 3 silver coated capillaries (column A, C and E) and uncoated capillaries (column B, D and F) loaded with four concentrations of fluorescein (rows 1-4), each in three replicas. The array was illuminated by a blue LED equipped with a blue excitation filter, and the array was analyzed with a green emission filter. The signals of the capillaries were detected by the CCD digital camera equipped with Tamron manual zoom CCTV 4-12 mm, f/1.2 lens. (6) The corresponding ImageJ 3D image of V. The fluorescein concentrations used: row #1: 100 nM, row #2: 10 nM, row #3: 1 nM, row #4 0.1 nM.