Towards a Point-of-Care Diagnostic System: Automated Analysis of Immunoassay Test Data on a Cell Phone

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ABSTRACT

Many of the diagnostic tests administered in well-funded clinical laboratories are inappropriate for point-of-care testing in low-resource environments. As a result, inexpensive, portable immunoassay tests have been developed to facilitate the rapid diagnosis of many diseases common to developing countries. However, manually analyzing the test results at the point of care may be complex and error-prone for untrained users reading test results by eye, and providing methods for automatically processing these tests could significantly increase their utility. In this paper, we present a mobile application that automatically quantifies immunoassay test data on a smart phone. The speed and accuracy demonstrated by the application suggest that cell-phone based analysis could aid disease diagnosis at the point of care.

Categories and Subject Descriptors

I.2.10 [**Vision and Scene Understanding**]: Intensity,color, photometry, and thresholding; I.4.8 [**Scene analysis**]; I.5.4 [**Applications**]: Computer vision

General Terms

Measurement

Keywords

Computing for development, point-of-care diagnostics, immunoassay, computer vision, mobile phone, smartphone.

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1. INTRODUCTION

Disease detection and epidemiology are limited by the scarcity of accurate, convenient and affordable diagnostic tests in the developing world. Many of the tests that are routinely administered in clinical laboratories are inappropriate for point-of-care (POC) settings, where low-income patients may be most accessible. Successful POC diagnostic tests are typically portable and produce rapid and accurate results without requiring significant user intervention or training. They cost little to produce and administer, and incorporate reagents that are stable without refrigeration. Biochemical immunoassay tests address many of these challenges, and are capable of diagnosing a variety of diseases common to the developing world such as malaria, dengue fever and typhoid fever [13].

An immunoassay is a biochemical test that measures the concentration of a specific substance or analyte in a solution that may contain a complex mixture of substances. Inexpensive, disposable assay cards have been developed that contain all the reagents required to process a biological sample. A benefit of performing an immunoassay diagnostic test at the point of care is that the biological sample used for testing does not need to be refrigerated, protected, or transported. Furthermore, these tests often run rapidly. As a result, the test can be analyzed at the POC and patients can receive treatment immediately. This rapidity is advantageous because many rural patients travel long distances to reach medical facilities and may be unable to return easily to collect test results, which could delay treatment [12].

While the benefits of immunoassay tests for POC diagnostics are clear, reading and interpreting the optical signals produced by the tests can be challenging. Manually analyzing the immunoassay signals can be a complex and error-prone process, particularly for untrained users attempting to read the results by eye. The test must often be read at a precise time after the biological sample is added; failing to do so could result in an unusable or incorrect result. Additionally, rather than simply identifying a positive or negative test result, users are frequently required to quantify immunoassay signals to determine the amount of analyte present in the sample. For example, in the CD4 rapid test, used to decide when to begin treatment of HIV infection, the intensity of the assay capture line may indicate whether a patient quantifies for antiretroviral treatment [2]. Furthermore, monitoring changes in assay signals over time, rather than using a simple endpoint measurement, enables improved timing of measurements and an ability to validate correct operation of a test [10]. The complexity of accurate immunoassay test analysis suggests that the utility of these tests could be increased by providing a method for automatically interpreting test results at the POC.

A variety of technologies could be considered when designing a tool to automate interpretation of immunoassay test results. The ubiquity, increasing processor power, and decreasing price of smart phones make them an attractive option, particularly for low-resource environments. Smart phones are portable and battery-powered, and may therefore be used in areas without electricity. Many smart phones have built-in cameras, GPS sensors, and network interfaces, making them a good platform for supporting a variety of medical applications. Furthermore, previous work by Stevens et al. has demonstrated that smart phone cameras are capable of capturing high-quality video of immunoassay tests [8]. However, this implementation requires that the video files be processed on a computer using software developed in MATLAB [5]. To utilize immunoassay tests effectively as a POC diagnostic tool, it is essential to provide a way to analyze results without additional complicating technology such as a computer and proprietary MATLAB software.

This paper presents a mobile application that automatically quantifies immunoassay data using image processing performed entirely on a cell phone. The remainder of the paper is structured as follows: in section 2 we discuss previous work that captures and quantifies immunoassay data. We present the system architecture and image processing techniques used to create our application in section 3 and evaluate our quantification methods in section 4. Finally, we discuss other work in this area in section 5 and our ideas for future work in section 6 before concluding in section 7.

2. PREVIOUS WORK

The methods for quantifying assay data presented in this paper build on work by Stevens et al. [8] who developed a low-cost, paper-based microfluidic flow-through membrane immunoassay (FMIA) device. On-card rehydration of assay reagents allows unrefrigerated storage of the device and provides several months of reagent stability. The data that is analyzed is from an assay that captures the progress of a test for a malarial biomarker. The presence of the biomarker causes the reagents on the assay card to change the color of a capture spot, and the rate and degree of color change indicate the quantity of biomarker present in the sample.

To measure the progress of the assay, a number of features were added to the device that facilitate video capture by a cell phone. The small flow-through area of the assay and smaller dimensions of the capture spots (less than 1 mm in diameter) require magnification to produce images of sufficient resolution for quantification. The magnification is provided using an off-the-shelf 15x simple lens (approximately $30). Additionally, an intensity standard printed with a conventional inkjet printer is attached to the card, which allows for normalized quantification of signals under variable lighting conditions. Finally, the assay is patterned with registration marks that may be used to locate the capture spot. The on-card intensity standard and registration marks, depicted in figure 1, simplify the task of automatically locating and processing the test result.

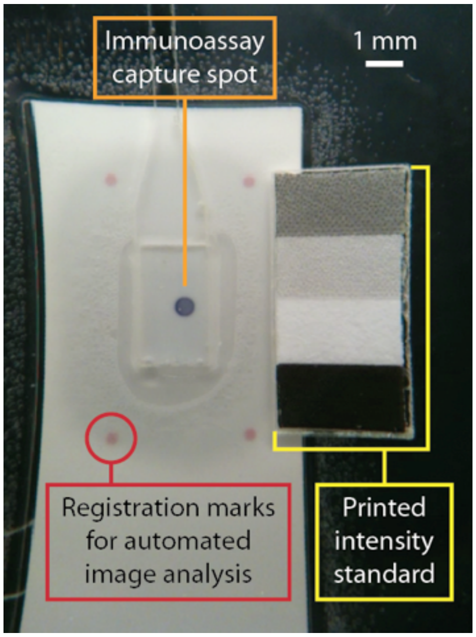

Immunoassay capture spot
1 mm
Registration marks for automated image analysis
Printed intensity standard

Figure 1: Device setup showing the location and scale of the on-card intensity standards, registration marks and immunoassay capture spot.

To record the progress of the assay, video was captured using an Apple iPhone 3GS with a resolution of 480x640 pixels per frame. Quantification of the assay results was performed by determining the intensity of the capture spot using pixel color intensities that are normalized to on-card intensity standards. The locations and intensities of the standards, registration marks and capture spots were identified using image processing MATLAB software written specifically for this task. The iPhone 3GS was used solely as an image and video capture device, and while this proved that this phone is capable of capturing images of sufficient quality, none of the image processing was performed on the phone.

3. METHOD

The main contribution presented here is the creation of software capable of automatically quantifying assay data using image processing performed on a cell phone. This section discusses the architecture of the application, the technologies used in the development of the system, the image processing algorithms implemented on the phone to quantify recorded assay signals, and the user interface that facilitates selection of assays for processing and displays the processed test results. The methods described here closely follow those presented in previous work, but leverage a number of existing optimized computer vision algorithms such as Canny edge detection [1] and contour-finding [9].

3.1 Architecture

The software for quantifying assay data was developed on the Android platform. We chose Android because of the

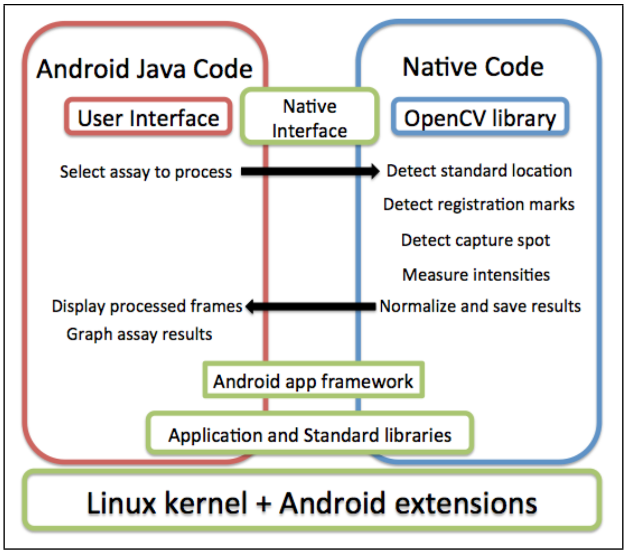


Figure 2: Application architecture and workflow.

variety of different devices that run that operating system including a variety of mobile phones and tablets, as well as the open-source nature of the development environment. Figure 2 shows the architecture of the application and how the application components fit together. The user interface and visual display of the quantified assay signals are implemented in Java using the standard Android Software Development Kit (SDK). To facilitate the use of optimized computer vision algorithms, the image processing components of our application make use of OpenCV, an open source computer vision library [11]. Since OpenCV is written in native code, we used the Android Native Development Kit (NDK) to compile the OpenCV library and our native application code for inclusion in the Android application. We then use the Java Native Interface (JNI) to access the native code. This allows us to take advantage of the convenience of the Android framework for the user interface and graphical display, but still make use of the optimized computer vision algorithms provided by OpenCV.

3.2 Image Processing

This section describes the algorithm used to automatically quantify video frames of an assay test. Processing every frame was found to be unnecessary since accurate quantification may be achieved using a small fraction of the total number of frames. Instead, frames were sampled at evenly-spaced intervals for the duration of the video. For each sampled frame, there were 4 main steps in the image processing algorithm: locating the on-card standards, locating the registration marks, locating the capture spot, and measuring the pixel intensities of the standards and capture spot.

3.2.1 Detecting the Intensity Standard

The first step in processing the assay is the identification of the on-card intensity standard. This determines the location of the standard measurement areas, and provides a means of estimating the location of the registration marks. A long, vertical strip through a portion of the image likely to contain the intensity standards is defined, and Canny edge detection [1] used to identify the horizontal edges of the standard. A similar process is used to identify the left and right edges of the standard. The identified image region is then subdivided into the four color standards, which are easily labeled as white, light gray, dark gray and black using simple color analysis. The measurement regions, used to calculate the intensity of the standard during the quantification process, are defined using a rectangular region that is slightly smaller than each intensity standard.

3.2.2 Detecting the Registration Marks

The next step in processing the assay is to identify the locations of the registration marks. The assay card design places the marks at known distances from the capture spot, and at specific locations relative to the intensity standard. The location of the standard, determined by the previous step, is used to estimate the four regions of the image that are likely to contain a registration mark. This is advantageous since it decreases the search space that must be considered to locate the registration marks. Within these estimated regions, the registration marks are red in color and highly saturated. This allows them to be segmented from the background image by thresholding on the hue, saturation and value channels. The thresholded channels are then combined to create a binary map of the image region, and contour detection performed to identify shapes that are likely to be the registration mark. The moments of each detected contour are used to calculate the area and centroid of the contour, and the registration mark is identified as the contour whose area falls within a known range. Finally, the centroid of the identified contour is taken to be the location of the registration mark.

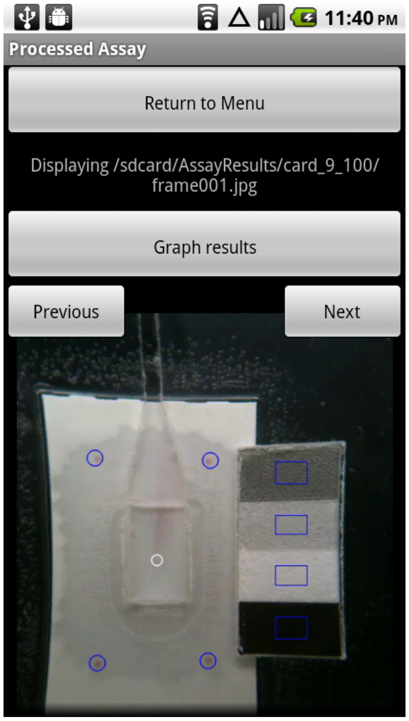
3.2.3 Detecting the Capture Spot

The capture spot is located at a known distance from the registration marks and is found as follows. First, half the length of the diagonal between two opposite registration marks is calculated. This length is used as the radius of a circle that is drawn around each registration mark, with the center of the circle corresponding to the centroid of the registration mark. The intersection of the four circles is then calculated, and the centroid of the intersecting area is taken as the location of the capture spot. The measurement area is defined as a circle of pixels within a fixed radius from the calculated center. The radius of the measurement area is chosen to be slightly smaller than the capture spot to avoid the possibility of taking measurements at the edges of the spot, which may incorrectly influence the intensity value. Our approach has the benefit that, if a registration mark fails to be detected, the capture spot location may still be correctly identified by the other three registration marks.

3.2.4 Quantifying the Assay Signal

The final part of the image processing is to measure and record the results of the biochemical assay. The mean pixel color intensity of each standard region (white, light gray, dark gray and black) is calculated, as well as the mean pixel color intensity for the capture spot area. The intensity of the capture spot is then normalized linearly to the white and dark gray standards, with the white standard corresponding to 0 and the dark gray standard corresponding to 1.

An image of each processed frame, as well as a file containing the locations of the standards, registration marks and capture spot, and both the original and normalized in-



Displaying /sdcard/AssayResults/card_9_100/frame001.jpg

Figure 3: Screenshot depicting displayed assay results with options to step through the processed frames or to graph the results.

tensity values, are created and saved on the SD card of the phone. The results of the assay may then be displayed to the user, sent away for further analysis or incorporated into data collection systems such as an electronic medical record.

3.3 User Interface

The user interface component of the application has been built using the standard Android application framework. When the application starts up, the user is presented with a list of image stacks from assays stored on the phone, and may choose a specific test to process. While the selected assay is being processed by the underlying native code, the user is presented with a "processing" icon to ensure that the user knows the application is busy and should not be interrupted. When processing is complete, the first frame of the processed image stack is displayed on the phones screen, shown in Figure 3, and the user can check the detected locations of the standards, registration marks and capture spot. The user can also step through the processed test on a frame-by-frame basis to observe changes in capture spot intensity. Alternatively, a "graph results" button is provided that, when clicked, displays a graph showing changes in the normalized assay signal and standard intensities for the duration of the test. Developing an intuitive and robust user interface suitable for non-expert users will be an extremely important component of the application in a deployment and is discussed further in section 6 of this paper.

4. EVALUATION

We evaluate the performance of the mobile application by comparing the results of processing to those of the previous MATLAB software [8]. The MATLAB implementation was run on an Apple MacBook Pro with a 2.53 GHz dual core processor and 4GB RAM, while the mobile application was run on two different Android devices, a Motorola Droid and an HTC Nexus One, both running Android 2.2.1. The Droid has a 550MHz processor, 256 MB RAM, a 16GB SD card and a 5.0MP camera, while the Nexus One has a 1GHz processor, 512 MB RAM, a 4GB SD card and a 5.0MP camera.

4.1 FMIA Data Set

To compare the image processing on the Android phone with the MATLAB software on a computer, we analyzed the same data set as used by Stevens et al. [8]. At this stage we have not captured new video data using the camera on the Android phone, but have rather processed the existing video data captured previously using the camera on an Apple iPhone 3GS. Our plans for facilitating the capture of new data using an Android phone are outlined in section 6.

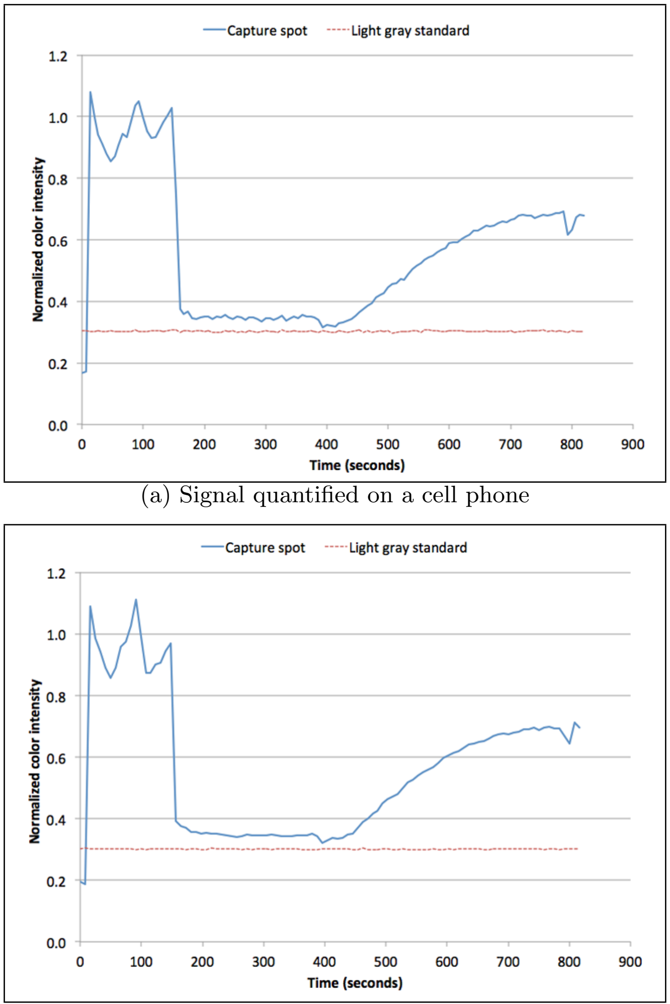
The data captured was from an immunoassay that models an assay format used to test for malaria, dengue, measles, and rickettsia [13]. In these immunoassays, the presence of certain antibodies in the sample causes the capture spot to turn red, and then to become darker and bluer with the addition of a gold-enhancement solution. The darkness of the capture spot increases with increasing concentration of antibodies in the sample. The changing color of the capture spot is recorded over time, and the rate at which this change occurs is calculated and used to quantify the assay signal.

Measuring the rate of change in assay signals over time is a commonly accepted improvement over single endpoint measurements for several reasons [10]. Firstly, since the concentration of analyte present in a given sample is unknown, it can be difficult to determine the appropriate endpoint at which to record the result. Measuring the result too early may prevent low-concentration samples, whose signals have not yet risen to the appropriate level, from being accurately recorded. However, measuring the result too late can cause inaccuracies due to a variety of factors, such as a build up of reaction products or depletion of substrate. Additionally, automatically detecting and identifying predictable events during the course of an assay can provide timing cues for operation and analysis, such as when to record a measurement [3]. Finally, kinetic data allows user errors to be identified early and the resulting test data discarded.

The data set we analyze consisted of 20 videos, each of which recorded the progress of a single assay. The videos ranged in length from 13:14 minutes to 13:52 minutes and were captured at 30 frames per second with a resolution of 640 by 480 pixels per frame. Processed frames were typically sampled at a rate of one out of every 200 frames evenly spaced throughout the duration of the video, which resulted in processing an average of 122 frames per test.

4.2 Accuracy

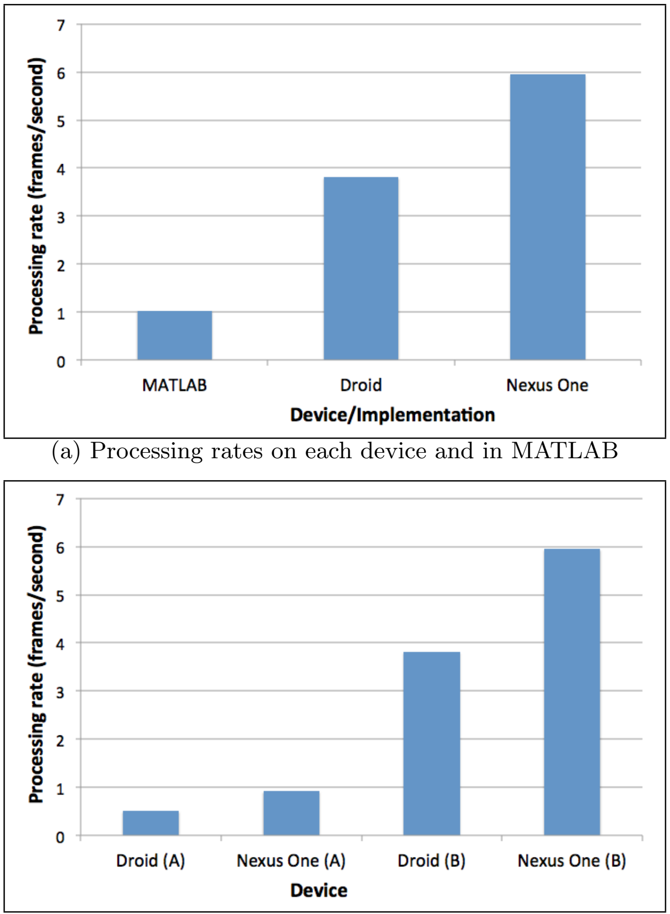
The mobile application correctly located the intensity standards, registration marks and capture spot on all 20 test videos attempted, and successfully extracted signal intensity measurements from every assay. We compared the quantitative measurements obtained by the mobile application, depicted in figure 4(a), to those obtained by the MATLAB implementation, depicted in figure 4(b). The shape of the curve representing the capture spot describes the progress of the assay test. The initial spike is due to the addition of



(a) Signal quantified on a cell phone

(b) Signal quantified using MATLAB

Figure 4: Normalized signal intensities over time: (b) using MATLAB and (a) using a cell phone



(a) Processing rates on each device and in MATLAB

(b) Processing rates on each device when (A) calculating locations every frame and (B) calculating locations on the first frame only

Figure 5: Comparison of performance on different Android devices and using MATLAB

a colored labeling reagent. This is then washed out, leaving behind the spot intensity. The subsequent increase in darkness of the spot is due to the addition of gold-enhancement solution. The line representing the intensity of the light gray standard is provided for comparison, and correctly remains constant for the duration of the test. The small differences between the two graphs may be attributed to the different methods used to identify the pixels corresponding to the capture spot area. Additionally, the exact frames that were sampled by each implementation varied slightly. However, the general similarity of the graphs indicates that the assay results obtained by the mobile application are comparable to those of the previous method, and that the application is capable of accurately quantifying immunoassay test results on at least two Android phones.

4.3 Processing Rate

We evaluate the rate at which assay data is processed with respect to several different factors. First, we compare the rates of processing assay data on the Android devices with the computer-based MATLAB software. For these tests, the locations of the standards, registration marks and capture spot were calculated only for the first frame and applied to each subsequent frame. The results of the comparison, depicted in Figure 5(a), show that the Nexus One achieved the fastest processing rate, averaging 5.95 frames per second (fps). The Droid achieved 3.81 fps and the MATLAB algorithm 1.03 fps. The superior performance of the mobile application may be attributed to the efficiency of implementing the image processing in native code and indicates that the application would be capable of processing assay data fast enough for the results to be available to health workers and patients within a single patient visit.

Second, we compared the processing rate on the Nexus One and the Droid when the calculations to determine the location of the capture spot, registration marks and standards were performed every frame, figure 5(b)(A), rather than only on the first frame, figure 5(b)(B). Performing all of the location calculations for every frame resulted in a processing rate of 0.92 fps on the Nexus One and 0.51 fps on the Droid, whereas calculating the locations for only the first frame resulted in an overall processing rate of 5.95 fps on the Nexus One and 3.81 fps on the Droid. The benefit of performing the location calculations for every frame is that the algorithm may accommodate slight movements of the phone during video capture. However, the length of the test necessitates the use of a mount to hold the phone securely, which makes such movements unlikely. As a result, performing these calculations only once per test is a valuable optimization and yielded signal curves that were visually indistinguishable from those generated by calculating the locations for every frame.

5. RELATED WORK

Several recent projects couple cell phones with biochemical assays. Skannex [7] markets a lateral flow assay system in which each assay is marked with an identifying barcode. An image of the test is captured and sent to Skannex for processing. Similarly, Martinez et al. [4] describe a prototype system for quantifying paper-based bioassays. The assay data is captured with a cell phone camera and sent to off-site medical experts for analysis. The disadvantage of these approaches is that they require an established communications infrastructure for transferring the assay data to an off-site laboratory for analysis by trained experts. In contrast to this, Matthews et al. [6] developed a paper dengue test that can be imaged and processed by a smartphone. The test creates a color on the paper, and a single image is captured of the test result and processed by the phone, which quantifies the color levels by comparing them with reference colors. The approach taken is not capable of measuring kinetic test data, which makes our application more flexible in measuring both the final intensity of the capture spot and the progress of the test over time, allowing more discriminating measurements to be made.

6. FUTURE WORK

There are a number of directions for future work that we intend to pursue. Firstly, we plan to test an experimental approach for capturing assay data using the camera on an Android phone. Additionally, we aim to quantify the assay signal as the test is run, rather than first storing a video of the entire test. Using this technique, the assay could be quantified in no more time than it takes to run the test.

Secondly, it will be interesting to investigate the potential for the phone to process a variety of different types of assays beyond the model assay used here. Extending the application to accommodate tests for a variety of diseases would make it a more powerful diagnostic tool. It would also be useful to enable the test results to be communicated over a cellular or 3G network and combined with other patient data, such as an electronic medical record.

Lastly, our initial implementation provides a simple user interface that highlights the functionality of the application. Currently, the test result is delivered as a graph of normalized capture spot intensities, which is not useful for patients or health workers. To address this, a method is required that maps the quantified data into a meaningful diagnosis that may be used to advise patient treatment. Furthermore, a deployment of the application would require careful user interface design to ensure that it would be a usable diagnostic tool for non-expert users in low resource environments. In addition, obtaining approval for the application as a medical device is also an important future consideration.

7. CONCLUSION

To be appropriate for use at the point of care, diagnostic tests must be portable, require minimal user intervention and training, incorporate reagents that are stable without refrigeration, cost little to produce or run, and give rapid and accurate results. This paper presents a mobile application for automatically quantifying low-cost, portable immunoassay tests using image processing performed entirely on an Android phone. The speed and accuracy exhibited by the application suggest that it is capable of delivering accurate diagnostic results immediately to patients and health workers in low resource environments.

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