Smartphone-based lateral flow imaging system for detection of food-borne bacteria

Youngkee Jung¹, Yoojung Heo², Jaejoong Lee³, Amanda Deering², and Euiwon Bae¹*

¹Applied Optics Laboratory, School of Mechanical Engineering, ²Department of Food Science, ³Department of Computer Science, Purdue University, West Lafayette, Indiana 47907, USA

*Corresponding author: ebae@purdue.edu

Abstract: We report an application for the smartphone as an accurate and unbiased reading platform of a lateral flow assays for food safety application. In particular, this report focuses on detection of food-borne bacteria in samples extracted from various food matrices. The lateral flow assay is a widely accepted methodology owing to its on-site results and low-cost analysis, even though sensitivity is not quite equivalent to that of standard laboratory equipment. An antibody-antigen relationship is transduced into a color change on a nitrocellulose pad; interpretation of this color change can result in uncertainty, particularly near the detection limit of the assay. Employing the high resolution integrated camera, constant illumination from light source, and computing power of a smartphone, we provide an objective and accurate method to determine the bacterial cell concentration in a food matrix based on the regression model from the color intensity of test lines. A 3D-printed sample holder was designed for representative commercial lateral flow assays and an in-
house application was developed in Android Studio to solve the inverse problem to provide cell concentration information from the color intensity. Test results with *E. coli* O157:H7 as a model organism suggests that smartphone-based reader can detect $10^4$-$10^5$ CFU/ml from ground beef and spinach food matrices.

OCIS codes: (330.1730) Colorimetry; (150.1708) Color inspection; (280.1415) Biological sensing and sensors; (120.0120) Instrumentation, measurement, and metrology

http://dx.doi.org/10.1364/AO.99.099999

### 1. Introduction

The lateral flow assay (LFA) is a widely used analyte-detection platform such as pregnancy tests, chemical residue determination, and toxin detection (Anfossi et al., 2013). The strength of the LFA is that detection can be performed on site; the cost of analysis is very low, since users need only to read out the presence or absence of certain color lines. These color lines are typically designed to capture target analyte and using a specific antibody and antigen relationship. In addition, some form of nanoparticles is used to generate a distinctive color, which is caused by the surface plasmon effect (Hsieh et al., 2017, Ngom et al., 2010). LFA has also been used for detection of microbial contamination ranging from *Bacillus* (Posthuma-Trumpie et al., 2009, Sajid et al., 2015), *Legionella* species (Posthuma-Trumpie, Korf and van Amerongen, 2009), *Enterobacter* (Sajid, Kawde and Daud, 2015), and *E. coli* O157:H7 (Fisher et al., 2009, Gumustas et al., 2018) to *Listeria monocytogenes* (Gumustas, Caglayan, Eryilmaz, Suludere, Soykut, Uslu, Boyaci and Tamer, 2018). One of the limitations of LFA detection is that reading the test line depends on the user’s eyesight and can become challenging, particularly when the analyte concentration is close
to the detection limit of the assay and interpreting colors in a different illumination conditions. In addition, since color intensity cannot be accurately quantified, the reported outcome is simple binary information, and analyte concentration cannot be retrieved in a reliable manner. Nowadays, the smartphone has become a necessity of everyday life. Smartphone handsets have become widely available, and their cost dramatically decreased owing to emergence of a multitude of companies, while the performance specifications continue to improve. These favorable features make the smartphone more than a simple communication device. Characteristics such as large random access memory (RAM), high-speed central processing unit (CPU), high-resolution complementary metal oxide semiconductor (CMOS) sensor, and wi-fi network are similar to the modern-day computers needed for laboratory-based testing. Now these features enable the smartphone to be the basis for a field-deployable instrument. One requirement for this application is to design an optical attachment that can convert the color information into analyte concentration so that default smartphone cameras can measure the concentration of the sample of interest. Many authors have explored this idea and introduced diverse attachments that transduce the signal of interest (Geng et al., 2017, Kılıç et al., 2018, Pohanka, 2017, Rasooly et al., 2016, Shin et al., 2018). Smartphones have been transformed into microscopes (Kim et al., 2013, Wareham et al., 2016), colorimetric devices (Jung et al., 2015, Kılıç, Alankus, Horzum, Mutlu, Bayram and Solmaz, 2018, Pohanka, 2017, Suria et al., 2015, Wang et al., 2017), luminometers (Arpa et al., 2012, Kim et al., 2017, Switz et al., 2014), and spectrometers (Das et al., 2016, Jung et al., 2017, Wang et al., 2016, Zhang et al., 2016) Among those applications, we focus here on the design and application of the smartphone-based lateral flow assay (SLFA). The main design aim was to provide adequate illumination to the sample strip, design a sample container to hold the LFA strips, and develop an app that can quantify the color intensity in the region of interest.
Here we propose to use the smartphone as a colorimetric and quantitative reader, thereby improving classification of the minute changes resulting from different analyte concentration (You et al., 2013). This will be particularly important for lower-concentration samples where, depending on the color shades and illumination condition, the naked eye cannot confidently report the presence of a test band. In addition, smartphones record the digital image of the experimental result; time and date stamps are always recorded as well, a bonus for database purposes. Here we report the design, fabrication, and testing of smartphone-based LFA detection with special emphasis on the detection of *E. coli* O157:H7.

**2. Materials and Methods**

**2.1 Bacterial sample preparation**

*E. coli* O157:H7 was incubated in LB broth 18-19 h at 37 °C. The culture was diluted with 0.1 M phosphate buffer (pH 7.0) and a concentration of $10^4$ CFU/ml was used to test the strips. To test the detection limit, each culture was diluted with 0.1 M phosphate buffer (pH 7.0), and concentrations of $10^4$, $10^5$, and $10^6$ CFU/ml were used to test the strips. To test specificity, *E. coli* O157:H7, *Salmonella typhimurium*, *E. coli* K-12, and *E. coli* 99-1044 A1 were incubated in Luria-Bertani (LB) broth, and *Listeria monocytogenes* was incubated in Brain Heart Infusion (BHI) broth for 18-19 h at 37 °C. For food-spike testing, *E. coli* O157:H7 and *Salmonella typhimurium* were incubated in Luria-Bertani (LB) broth 18-19 h at 37 °C. Each culture was diluted with 0.1 M phosphate buffer (pH 7.0); For the food test, 25 grams of spinach and 10% lean ground beef of sample was used and $10^3$, $10^4$, $10^5$ CFU/ml of *E. coli* was added to each sample. After addition of 225 ml of 0.1 M phosphate buffer the homogenized liquid sample was used to test the strip. 1 mL of non-inoculated samples and *Salmonella*-containing samples were used as negative controls. For
each strip, the procedure followed the instructions that came with the kit (Romber-Labs, 2019). Test results were imaged with the cell-phone camera application.

2.2 Imaging attachment

The core of the smartphone-based imaging system relies on three major sub-components: sample cartridges for each brand of LFA strip, interchangeable optical imaging box, and smartphone cradle (Figure 1). Sample cartridges were designed to accommodate different strip dimensions to be imaged within the camera imaging area and repeatable lateral positions (Figure 1(A)). The optical imaging box, which is an interchangeable module, was designed to be inserted into a smartphone cradle (Figure 1(B), (C)). Smartphone cradles were designed separately for two different models tested (OnePlus One, model A0001, Shenzhen, China, and LG G4, model H811, Seoul, Republic of Korea) since their overall dimension are different.

Figure 1. Photo and schematic diagram of the overall smartphone based lateral flow imaging system. (A) Sample holder that was designed to match the dimension and location of the control and test band. (B) Optical imaging box that provide diffusive illumination and magnified image of the control and test band area. Dimension of the box is
2.5 x 3.8 x 2 cm. (C) Smartphone cradle that holds the phone in horizontal direction. Wire diagram in the middle shows schematics of overall assembly.

Detailed design of the proposed optical imaging box is shown in Figure 2(A). This box consists of a reflector to redirect the smartphone LED light onto the strip. To avoid specular illumination, an optical diffuser was inserted to spread out the incoming LED light. Sample LFA was imaged with a plano-convex lens that relays the image to the smartphone camera lens. Figure 2(B) shows a photograph of the imaging box where an individual Solidworks CAD model is shown in Figure S1.

Figure 2. Schematic diagram of the lateral flow imaging box. (A) Smartphone LED light is re-directed to a diffuser so that strong specular illumination becomes more diffusive illumination. (B) Break-down of the imaging box with lens, 3-D printed case, reflection mirror, and diffuser.

2.3 Experimental procedure

Two different brands of LFA strips were used for this model study: *E. coli* O157:H7 rapid detection kit (Model ETLF-020, Bioassay, Ijamsville, MD, USA), Rapidcheck *E. coli* O157:H7 test kit 7000157 (Romer Labs, Newark, DE, USA). When bacterial samples were ready, each LFA strip was prepared according to manufacturer’s directions. Common preparation elements included
adding promoter from the kit and incubating the strip for approximately 15 min at 42 °C. After a 15-min cool down of strips, they were added to the appropriate individual cartridge and inserted into the smartphone for reading (Figure 3). The overall procedure was divided into three steps. First was conducting a standard dilution series test to acquire calibration data for color intensity versus bacterial concentration. Second was conducting a specificity test in which four different non-\textit{E. coli} O157:H7 samples were challenged against the LFA strip. Third was conducting a food-spike test in which two representative food matrices (ground beef and spinach) associated with \textit{E. coli} O157:H7 outbreaks were selected.

\textbf{Figure 3.} Sample preparation steps. (A) Approximately, transfer 200 uL of enriched bacterial sample to a sample cup and prepare the LFA strip. (B) Add 1 drop of promoter reagent (C) Place the LFA into the sample cup and incubate at 42 ± 1°C for 15 minutes. (D) Remove the LFA from the incubator and wait for 15 min. (E) Perform visual reading of control and test line.

\textit{2.4 Data analysis}

Native images from the phone were transferred to computer to be analyzed by Matlab scripts. First of all, original color RGB images were transformed to grayscale images and cropped to display only the LFA assay portion for each standard-concentration, specificity, and food-spike sample.
With the longer dimension defined as the x axis and the shorter dimension as the y axis, 2-D image intensity was spatially averaged in the y direction so that a 1-D linear intensity cross section was achieved. Utilizing Matlab’s curve-fitting toolbox, an 8\textsuperscript{th}-order polynomial function was used to model the background image from illumination and subtracted from the 1-D linear cross-sectional plot. The result shows two peaks (control and test line); both peak area and intensity were used to estimate the presence of the test line and concentration of the analyte. Since each brand of LFA strips had different locations and widths of the control and test lines, this process was separately optimized for each brand.

2.5 App development

To implement the whole functionality independent of a separate computer, an Android app was developed to implement similar processes and calculations on the smartphone. First off, the current app asks the user to select the brand of the LFA (one of the three tested) because the LFA strip area and respective control and test-line dimensions were coded into the app development. Once a brand is selected, the app automatically crops the white strip area and performs a spatial averaging calculation as explained in section 2.4. Second, a fitted curve is created using a polynomial curve fitter (Apache, 2019) with a degree-of -6 equation. Unlike Matlab’s curve-fitting function, this library required careful coding to make a better fitting around the peak (control and test) areas. In particular, signs of slope were checked on the each side of the peak locations. Data points were estimated using a polynomial curve fitter, if there were no dramatic changes in its slope. However, if there were such changes, those points were estimated using linear regression. For graphing the plot, a 3\textsuperscript{rd}-party library called GraphView was used (Android, 2019). When the first time the app is ran, the user is asked to provide calibration images for which bacterial samples of known CFU/ml are deposited on the test strip. After processing by the same procedure described above,
a linear calibration curve from control to $10^6$ CFU/ml is constructed. When an unknown sample is inserted in the phone, the app compares the intensity value to the calibration curve to provide an estimated CFU/ml. For the on-phone database, Sqlite for Android was used (Android, 2019), in which the app records the test-strip image, date, time, and test location. This information can be tagged to provide geographical location of the test result using default map software such as Google Map.

3. Results

3.1 Standard dilution series

**Figure. 4.** Core of the image processing and calibration step. (A) displays the concentration dependent LFA image taken with the proposed imaging attachment. Control line (left) is shown for every sample while test line (right) are less visible for lower concentration. (B) express the fitting function to remove the background reflectance from nitrocellulose paper. Top image shows the raw image which is 1-D average of the Y-direction of the LFA image. After
fitting the curvature with 8th order polynomial, the fitting function minimize the residue and provides flat response of control and test time.

The processing steps described in section 2.4 are shown in Figure 4. To provide a proof-of-concept for a concentration dependent LFA, captured images from the dilution series of *E. coli* O157:H7 sample were analyzed. Figure 4(A) displays bacterial concentration of $10^4$ CFU/ml to $10^6$ CFU/ml from the Bioassay LFA with the definition of Cartesian coordinate. Visual observation clearly detects the presence of a test band on the $10^5$ and $10^6$ CFU/ml samples, while the $10^4$ CFU/ml sample is not so certain. However, Figure 4(B) demonstrates that after curve fitting with an 8th-order polynomial and background subtraction, peak intensity from both control and test line was visible at approximately 1200 and 2300 pixels, respectively.

Figure 5. Result of the dilution series for Bioassay and Rapidcheck. (A) Comparison of the 1-D cross-sectional intensity for control (left) and test (right) lines. (B) shows the integrated intensity versus log CFU number with it’s
fitting function ($R^2=0.992$) for Bioassay (C) shows the similar results for Rapidcheck with (D) fitting function ($R^2=0.997$).

Figure 5 shows the dilution series test for the two different brands of LFA for $10^4$, $10^5$, and $10^6$ CFU/ml. Direction of flow is from right to left. The Bioassay LFA displayed sharp and narrow peaks with asymmetric peak shape, whereas the other two brands show more symmetric peaks for both control and test lines. Individual brands showed different peak intensities depending on the concentration changes. Based on these results, intensity of the test bands from Bioassay with similar sample concentrations resulted in better contrast than that of the Rapidcheck. To provide quantitative assessment of the performance, average integrated intensity and standard deviation were calculated for the test line as shown in Table S1 which shows detection limit of $10^4$ CFU/ml for Bioassay and $10^5$ CFU/ml for Rapidcheck. Original images are tabulated on Figure S2.

3.2 Specificity test

To test the possibility of false positives from the assays, a specificity test was conducted. Four non-$E. coli$ O157:H7 samples ($E. coli$ 99-1044 A1, $E. coli$ K12, $L. monocytogenes$, and $Salmonella$ typhimurium) were challenged; most of the strips provided good specificity. However, for the Rapidcheck LFA, $E. coli$ 99-1044 A1, $E. coli$ K12, and $Salmonella$ typhimurium samples were detected with a positive test line that was not visually observable owing to weak intensity (Figure S3) but was picked up by the image analysis. $L. monocytogenes$ did not provide any response on the same strip.
Figure 6. Result from specificity test using four different non-*E. coli* O157:H7 samples for (A) Bioassay and (B) Rapidcheck. 1-D cross-sectional intensity profile reveals that most of the strips provided good specificity while Rapidcheck *E. coli* 99-1044 A1, *E. coli* K12, and *Salmonella typhimurium* generated weak false positive response from the test line. This line was not easily observable by eye but smartphone imaging system can detect them.

3.3 Food-spike test

Ground beef and spinach artificially inoculated with *E. coli* O157:H7 were tested using LFA. Figure 7(A-B) displays the results from ground beef samples for Bioassay and Rapidcheck assays, respectively. All samples displayed positive lines for $10^4$ and $10^5$ CFU/ml. Similar results were found for spinach (Figure 7(C-D)). In this case, all brands provided positive lines for $10^5$ CFU/ml samples, while only the Bioassay LFA resulted in a positive line for $10^4$ CFU/ml. Original image was tabulated in Figure S4.
Figure 7. Food spike test results for ground beef samples with varying concentration of inoculum. (A) Bioassay (B) Rapidcheck. Similarly, spinach were inoculated with E.coli O157:H7 and challenged with (C) Bioassay and (D) Rapidcheck.

3.4 App development
Figure 8. Screenshot of app developed for lateral flow analysis with smartphone. (A) shows the first screen where user can select calibration, test, and access the database menu. (B) shows the screen for calibration process. Different vendors can be added for separate calibration (C) During the calibration, each concentration of cell numbers in CFU/ml is plotted for control and test line. Their average pixel values are integrated and saved. (D) shows the result of test function when new image is loaded. Based on the linear calibration plot, app reports the estimated concentration in CFU/ml. (E) Screenshot of the history function where thumbnail image, brand, date, time, and geographic location is saved. (F) Once one of the data entry is clicked, map software automatically displays the location where the data has been acquired.

A screenshot of the developed app is shown in Figure 8. At first, users can select calibration, test, and history as a sub-menu. The calibration process is required only once to acquire standard concentration images to provide estimated concentrations from unknown samples (Figure 8(A)). Then users are asked to select the brand of LFA as their respective control and test line positions.
and strip dimensions differ (Figure 8(B)). Figure 8(C) displays the internal curve-fitting process and plotting routing, while Figure 8(D) displays the result from an unknown sample interrogation. Based on the previously conducted calibration routine, the app estimates the bacterial concentration by CFU/ml and reports the result. Once the test is conducted, all of data are stored in a local database (Figure 8(E)) in which time, date, image, and location information are recorded. If the user clicks one of the listed items, any linked map software displays the geographical location of the test site (Figure 8(F)).

4. Discussion

One of the important design parameters for smartphone-based imaging system is the ability to provide consistent and repeatable imaging of samples. Diffusive imaging provided better illumination and less specular reflection of white LFA strips and a plano-convex lens enough magnification to provide a sufficient number of pixels to cover the control and test areas. The design of the sample cartridge has an extended dimension at the end so that when users push the cartridge into the imaging box, the sample is always positioned at the center of the camera image. In addition, different brands of phone typically require separate cradles; designing the imaging box as an interchangeable module provides consistency.

A critical component of peak analysis was to ensure differentiation of the background (blank) strip and positive test line. Even with the diffuse imaging technique, some degree of uneven illumination across the horizontal direction still exists, and it is critical to not modify the peak intensity or location and to subtract only the illumination effect. This aim was achieved by a baseline subtraction method, which is a widely accepted processing technique in other spectrum-based methods such as Raman spectroscopy. In Raman spectroscopic data, fluorescence background is present along with the native Raman spectrum; this background signal is typically
removed so that only Raman peaks are left for analysis. Similarly, LFA images with illumination generates a consistent background signal from the white nitrocellulose pads and this signal was treated like the fluorescence background of a Raman spectrum.

In terms of assay sensitivity, tested brands showed different CFU/ml detection limits. While tested brands claim to have a detection limit of $10^4$ CFU/ml after enrichment, our current test protocol did not include sample enrichment for any of the three tests conducted (standard concentration test, specificity test, and food-spike test). Even without an enrichment step for *E. coli* O157:H7, two brands have shown clear visual test bands for $10^5$ and $10^6$ CFU/ml. It is interesting to note that a smartphone-based LFA system can provide more consistent readings for samples close to the detection limit of the assays. In addition, each brand of assay must have proprietary design parameters in types of antibody, width of the test lines, type and size of gold nanoparticle used, and construction of the flow strip. This was confirmed by variations in the width of control and test peaks, slope of peaks, and location of peaks. For example, Bioassay tests have shown asymmetric peaks when flow is moving from right to left. The first area that contacts an incoming flow has a steep slope for intensity that gradually decreases. Based on the series of tests from Figure 5, sensitivity of each assay without enrichment Bioassay was more sensitive than Rapidcheck. Lastly, the intensity of the test band was roughly proportional to the CFU/ml; this was used as the basis for the calibration curve for the Android app development. For specificity, most of the samples tested showed no reaction from non-O157:H7 samples, but several test strips from Rapidcheck resulted in false-positive results. As noted in Figure S3, visual inspection does not perceive this false positive result, whereas smartphone-based LFA sensitivity detects minute color changes in the test band.
For food-spike testing, the matrix effect due to particulates from the food itself can adversely interfere with the detection limit of the LFA. In particular, testing with spinach samples visually colored the lateral flow strip green and overall negatively affected the detection limit, as shown in Figure 7 (C-D). All brands generated less intense peaks from the test bands with this sample matrix compared to the ground beef matrix. This result suggests a need for additional sample preparation to filter out large particulate from the matrix to maintain detection limits similar to those of standard pure-sample testing.

5. Conclusions

As an application of internet-of-things in research field, smartphone-based LFA reader provides various advantages compared to visual interpretation. The proposed method can provide unambiguous determination of the positive results especially for low concentration samples, interprets the color intensity to provide quantitative cell concentration information, and records digital image and geographic information of the LFA for future analysis and record-keeping. Future plans include applying this concept to different types of bacterial species such as *Salmonella* or *Listeria*, interrogating the same images with different color spaces such as YUV and L*a*b* (Kim et al., 2017), and increasing the dynamic range by utilizing the 10-bit digital-negative (DNG) format which has recently become available on most smartphones.

6. Acknowledgment

This material is based upon work supported by the U.S. Department of Agriculture, Agricultural Research Service, under Project No. 8072-42000-077. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.
7. References

Apache, c.m.A., 2019. Polynomial curve fit.


Conflict of interest

All authors declare no conflict of interest.