Normalizing the Optical Signal Enables Robust Assays with Lateral Flow Biosensors

Jin-Ho Park, Eung-Kyu Park, Young Kwan Cho, Ik-Soo Shin,* and Hakho Lee*

ABSTRACT: Lateral flow assays (LFAs) are widely adopted for fast, on-site molecular diagnostics. Obtaining high-precision assay results, however, remains challenging and often requires a dedicated optical setup to control the imaging environment. Here, we describe quick light normalization exam (qLiNE) that transforms ubiquitous smartphones into a robust LFA reader. qLiNE used a reference card, printed with geometric patterns and color standards, for real-time optical calibration: a photo of an LFA test strip was taken along with the card, and the image was processed using a smartphone app to correct shape distortion, illumination brightness, and color imbalances. This approach yielded consistent optical signal, enabling quantitative molecular analyses under different illumination conditions. We adapted qLiNE to detect cortisol, a known stress hormone, in saliva samples at point-of-use settings. The assay was fast (15 min) and sensitive (detection limit, 0.16 ng/mL). The serial qLiNE assay detected diurnal cycles of cortisol levels as well as stress-induced cortisol increase.

INTRODUCTION

Lateral flow assays (LFAs) are increasingly adopted for on-site molecular testing. Based on a capillary sample flow through membranes, LFAs are fast, affordable, and simple to carry out with minimal user interventions.1−3 Such advantages have promoted the development of rapid assay kits with applications in disease diagnostics, food surveillance, drug testing, and environment monitoring.4−8 Most LFAs generate optical signal when their detection targets are present. The signal can be conveniently detected via visual inspection (e.g., naked eyes), although the interpretation is qualitative (yes/no) and can be ambiguous at low-target concentrations.9,10 Coupling LFA devices with dedicated optical readers enables quantitative measurements, which (i) minimizes subjective data interpretation and thereby improves the detection accuracy;11 (ii) produces information (e.g., severity of diseases) to guide the most efficient intervention;12 and (iii) facilitates monitoring the efficacy of treatment or remediation. Adding an extra detector, however, could offset LFA’s practical merits of on-site and equipment-free applications.3,13

Smartphones can be a powerful companion tool for LFAs. Smartphones are ubiquitous and equipped with high-end cameras, microprocessors, and wireless communication functions. These capacities can facilitate transforming smartphones into a portable detector and data logger, readily available to LFA users. Proving the concept, smartphone-based LFA readers have been demonstrated, some of which were integrated with custom apps for data analyses.15−19 The following aspects, however, make it difficult to obtain reproducible assay results: (i) color imbalance under different illumination conditions; (ii) camera optics that vary among phone brands and change with hardware update; (iii) image correction by phones’ own proprietary algorithms; and (iv) geometric variations (e.g., camera angles and distance) caused by users. Attaching a separate dongle to a phone can address some of these challenges (i.e., illumination and alignment), but this solution falls back to the requirement of auxiliary hardware and still faces phone-specific camera issues.20−22 The phone-specific camera issues can be resolved when gamma correction is known. Indeed, removing gamma correction in acquired images produced colorimetric absorbance linearly proportional to the concentration of light-absorbing sources,23,24 which facilitated accurate quantitative assays. Unfortunately, gamma correction is often proprietary and inaccessible, and estimating gamma correction would require measuring camera’s spectral responses to illumination of varying wavelength.23,25

Here, we report a general strategy for accurate LFA signal detection via smartphone. Termed qLiNE (quick light normal-
It measures consistent LFA signal through real-time calibration of an imaging setup. To achieve this capacity, qLiNE used (i) a reference card (4 × 5 cm²) that accompanies LFA test strips and (ii) a customized app for image analyses. The card was printed with a quick response (QR) code, color standards, and alignment marks for LFA-strip placement. After taking a picture of the card and an LFA strip, the app set the in-photo spatial coordinate by recognizing the QR code, adjusted color space, and scanned the LFA strip. We tested qLiNE by measuring color signals from gold nanoparticles (AuNPs) on LFA strips. qLiNE compensated for different ambient light conditions (i.e., sunlight, dark room, fluorescent lighting, and yellow lighting), imaging angles, and camera versions; it thereby generated uniform signal and enabled quantitative measurements, all without requiring additional hardware. As a potential application by general users, we adapted qLiNE to detect salivary cortisol (CTS), a known stress hormone. The qLiNE test was fast (15 min), robust to imaging conditions, and sensitive, with the detection limit (0.16 ng/mL) below normal CTS levels (>2 ng/mL) in saliva.26 Serial monitoring further confirmed CTS diurnal rhythm (i.e., peaking in the morning then declining throughout the day) as well as stress-induced CTS increase.

### MATERIALS AND METHODS

#### Materials

- Hydrocortisone 3-(O-carboxymethyl)oxide (CS-3-CMO, 98%), bovine serum albumin (BSA, 99%), gold nanoparticle (AuNP, 20 nm), cortisol (CTS, C-106), polyvinylpyrrolidone (PVP, 10 kD), Tween-20 (TW-20, 1.228 kD), and anti-mouse IgG antibody were purchased from Sigma-Aldrich.
- SuperBlock blocking buffer (SB), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC, ≥98%), 20× borate buffer (pH 8.5), 10× phosphate-buffered saline (PBS, pH 7.4), and CTS enzyme-linked immunosorbent assay (ELISA) kit were obtained from Thermo Fisher Scientific.
- Nitrocellulose (NC) membrane (cat. #78316407) was purchased from Cytiva.
- Surfactant 10G (S-10G, 30−40 dyn/cm surface tension) was obtained from Fitzgerald.
- Anti-CTS antibodies were purchased from LSBio (LS-C79813-1), Abcam (ab1949), and Fitzgerald Industries (10R-C145A and 10−1546).

#### Synthesis of the CTS–BSA Conjugate (CTS<sub>BSA</sub>)

We mixed CS-3-CMO (50 μL) in ultra-pure water with EDC (0.5 M, 50 μL) and let the mixture react for 2 min at room temperature (RT). The activated CS-3-CMO was then mixed with BSA (5%, 200 μL) to form CTS<sub>BSA</sub>. After 1 h incubation, we triple washed CTS<sub>BSA</sub> via centrifugation (14,000 rpm) using a

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**Figure 1.** Overview of the qLiNE approach. (A) qLiNE was designed to produce reliable, high-precision results for LFAs. It auto-corrects optical signals by adjusting for ambient light conditions, color mixing, camera settings, and image distortions. (B) A qLiNE reference card is imaged together with an LFA test strip. The card contains a QR code, color pads, and a mounting space for the test strip. (C) Photo of a qLiNE assay system. A smartphone takes a photo of the qLiNE reference card and an LFA test strip, extracts corrected assay results, and uploads data to a cloud server. No additional auxiliary hardware is required. (D) Screenshots of a qLiNE app customized for cortisol detection. (Left) The app showed an imaging guiding box. (Middle) From the processed LFA image, the app read the signal intensity and estimated CTS concentration by referring to an internal lookup table. (Right) The measured data were stored in a history page.
centrifuge filter (3 kD cutoff; MilliporeSigma). The purified conjugates were aliquoted under N₂ gas charge and stored at −20 °C.

Preparation of the Antibody–AuNP Conjugate (Ab@AuNP). We triple washed anti-CTS antibodies (1 mg/mL, 50 μL in 1× PBS) via centrifugation (14,000 rpm) using a centrifuge filter (3kD cutoff; MilliporeSigma). We then added the purified antibodies (1 mg/mL, 10 μL) into 20 nm AuNP solution (7 × 10¹¹ particle/mL, 1 mL) containing 10 mM borate buffer (pH 8.5). The mixture was incubated for 1 h at RT, and then, BSA (5%, 50 μL) was added to block the unreacted AuNP surface. The mixture was incubated for additional 45 min at RT. Finally, we collected antibody-modified AuNPs (Ab@AuNPs) via centrifugation (12,000 rpm, 30 min, three times).

Preparation of an LFA Strip. The LFA strip had three components: a backing card, an NC membrane, and an absorption pad. We first attached an NC membrane (cat. #78316407, Cytiva) on an adhesive backing card and then placed an absorption pad at the end of the NC membrane with a 2 mm overlap. We next cut the assembled card into strips (0.4 × 4 cm²). Prepared test strips were stored in a desiccator at RT until use.

CTS Detection with a qLiNE-LFA. We spotted the CTSBSA conjugate (1 mg/mL, 1.65 μL) and anti-mouse IgG antibody (0.5 mg/mL, 1.65 μL) on the control and the test zones on an NC membrane, respectively. The spotted membrane was dried in a desiccator at RT (20 min). For CTS detection in buffer, we diluted Ab@AuNP stock solution (7 × 10¹¹ particle/mL) by 100-fold in a working buffer (0.5% PVP, 0.25% S-10G, and 0.25% TW-20 in 1× PBS) and then added CTS. For salivary CTS detection, we diluted Ab@AuNP stock solution (7 × 10¹¹ particles/mL) by 10-fold in a working buffer. Saliva spiked with CTC was then mixed with Ab@AuNP solution at 1:9 volume ratio (Ab@AuNP solution vs saliva). We dipped the end of an LFA strip into the prepared mixture. After 15 min, the reacted test strip was placed on the qLiNE reference card, and an image

Figure 2. Image correction with qLiNE. (A) Sequence of the qLiNE operation. (i) Three corner boxes in the QR code were recognized, and their locations were used to set the coordinate in a photo. (ii) Illumination was corrected in reference to the white and the dark color pads. (iii) Color imbalance was adjusted by calibrating red, green, and blue values, each from one of the three color reference pads. (iv) Last, the color signal on an LFA test strip was scanned along the membrane. (B) Photos of the strips imaged under different illumination settings: natural light, no light, fluorescent light, and yellow light. The signal intensities (inside a dashed rectangle) reflected the amount of membrane-bound Ab@AuNPs. Raw intensity values were difficult to compare each other among different illumination conditions (Figure S2). (C) qLiNE-processed images in (B) and generated signal intensities that environmental illuminations were corrected. For a given Ab@AuNP loading, the signal values became consistent, less affected by ambient light. Data are displayed as mean ± s.d. from technical triplicates. (D) LFA strips were imaged using a phone camera at different angles and distances, and the acquired images were adjusted by the qLiNE App. For a given AuNP concentration, the corrected intensity was statistically identical (one-way ANOVA) regardless of the camera positions tested. Data are displayed as mean ± s.d. from duplicate measurements.
was taken using smartphones (phone 1, Samsung Galaxy Z Flip3 5G SM-F711N and phone 2, Samsung Galaxy Note20 5G SM-N981U).

**CTS-Competitive ELISA.** We used a CTS-competitive ELISA kit (cat. #EIAHCOR, Thermo Fisher Scientific) as a gold standard for CTS quantification. In the antigen-binding phase, 50 μL of CTS, 25 μL of CTS-protein conjugate, and 25 μL of anti-CTS antibody were sequentially added to wells in a microtiter (96 wells) plate. After 1 h incubation at RT, the solution in each well was removed, and the well was washed five times with 1× wash buffer. Afterward, 100 μL of the 3,5,3',5'-tetramethylbenzidine substrate was added to each well (the solution turned to blue) and incubated for 30 min at RT. A stop solution was then added, and optical density (OD) at 450 nm was measured. To account for the competitive nature of the assay, the signal was normalized as \((S_0/S_1 - 1)\) where \(S_0\) is the baseline signal (CTS = 0 ng/mL) and \(S_1\) is the signal from CTS-present samples.

**CTS Monitoring in Saliva Samples from Volunteers.** We collected saliva samples from adults (over the age of 21) with consent. Following the standard process of saliva collection,27,28 we asked participants to rinse their mouth with water (one time). After rinsing, participants rested a few minutes and then placed an oral swab under the tongue for 1 min. Saliva was extracted from the swab by either centrifugation (10,000 rpm, 10 min) or squeezing. The extracted saliva was mixed with a concentrated working buffer (5% PVP, 2.5% S-10G, and 2.5% TW-20 in 10× PBS) at 9:1 volume ratio (saliva vs buffer). LFA test strips were immersed in each 40 μL mixture solution (working buffer + saliva + 7 × 10⁹/mL Ab@AuNPs). After 15 min, the reacted test strip was placed on the qLiNE reference card, and an image was taken using smartphones (Samsung Galaxy Z Flip3 5G SM-F711N or Samsung Galaxy Note20 5G SM-N981U). This study was approved by the Institutional Review Board (IRB) of Massachusetts General Hospital (IRB number 2019P003472; Principal investigator, Hakho Lee), and the overall procedures followed institutional guidelines.

**RESULTS AND DISCUSSION**

**qLiNE Approach.** Figure 1 summarizes the overall qLiNE concept. We designed qLiNE to recover colorimetric signals at high precision by correcting nonuniformity and systematic errors in optical imaging (Figure 1A). A key component is the qLiNE reference card (Figure 1B) which contains a QR code, color references, and a mounting guide for an LFA test strip. Users take a photo of the reference card along with an LFA strip (Figure 1C). A custom-designed qLiNE app then adjusts the image (e.g., shape distortion, illumination brightness, and color imbalances) based on the predefined patterns in the reference card and reads out the optical signal in the test strip. The qLiNE app also uploads raw images and processed data in a cloud server for further analyses (e.g., machine learning for image processing) or personal bookkeeping (see Movie S1 for app operation).

Figure 1D shows the snapshot of the qLiNE app designed for the CTS detection. The app was written in a programming language, Kotlin, using Android Studio and used OpenCV library for image processing. The app first displays the camera view overlaid with a guiding rectangle for image capture (orange edges in Figure 1D, left). Once an image is taken, the app extracts the normalized optical signal from a test strip and converts it to CTS concentration using an in-app lookup table (Figure 1D, middle). The data along with a timestamp is stored in the history page (Figure 1D, right), helping users track their CTS levels over time.

**Image Processing Algorithm.** The qLiNE image processing consisted of elemental recognition and signal conversion (Figures 2A and S1A). The app first searched for three corner squares on the QR code [Figure 2A(i)]. The location and the shape of these squares were used to calculate the photographing angle as well as to correct image skewness (Figure S1B). The app then read intensities from five color standard pads: red (R), green (G), blue (B), white (W), and dark (D). The intensity values \(W_i\) and \(D_i\), which were from W and D, respectively, were used to set the dynamic range \((T)\) for image brightness [Figure 2A(ii)]: \(T = W_i - D_i\). The app next calculated the color offset \((\Delta C)\) using RGB pads [Figure 2A(iii)]. For the red (r) channel, the offset was \(R_{0,r} - R_{s,r}\), where \(R_{0,r}\) is the predetermined reference value of the R pad and \(R_{s,r}\) is the measured intensity of the same pad in the r channel. Similarly, color offsets in blue (b) and green (g) channels were obtained using G and B pads, respectively.

\[
\Delta C = \left\{ \begin{array}{l}
R_{0,r} - R_{s,r} \\
G_{0,g} - G_{s,g} \\
B_{0,b} - B_{s,b}
\end{array} \right.
\]

In the signal conversion step [Figure 2A(iv)], the app read the raw optical signal \((L)\) of the LFA test strip in the red, green, and blue channels

\[
L = \begin{pmatrix}
L_r \\
L_g \\
L_b
\end{pmatrix}
\]

and made the channel-wise correction as \(L_c = L + \Delta C\)

\[
L_c = \begin{pmatrix}
L_{c,r} \\
L_{c,g} \\
L_{c,b}
\end{pmatrix} = \begin{pmatrix}
L_r + (R_{0,r} - R_{s,r}) \\
L_g + (G_{0,g} - G_{s,g}) \\
L_b + (B_{0,b} - B_{s,b})
\end{pmatrix}
\]

The corrected signal was then converted into the gray scale, \(L_g = (L_{c,r} + L_{c,g} + L_{c,b})/3\), which was finally background-subtracted and rescaled: \(L_g = 255 \times (L_g - D)/T\). Note that the qLiNE app was programmed to scan an LFA strip along its center line and find the maximum peak value in signal intensity; this method was consistently applied in all subsequent measurements. Placing the LFA strip along the printed alignment marks in a reference card made it easier to program the app to set LFA coordinates.

We evaluated the precision of the qLiNE algorithm by imaging LFA test strips with a reference card under different ambient light settings. Test strips were spotted with capture antibodies (anti-mouse IgG), and the ends of the test strips were immersed into buffer solutions of different Ab@AuNP concentrations. Photos of test strips were then taken under natural sunlight, no light, fluorescent light, and yellow light conditions (Figure 2B). Overall, the signal decreased with lower Ab@AuNP concentrations. However, the raw values at a given Ab@AuNP dose were significantly different among illumination conditions (Figure S2A) with the coefficient of variation (CV) > 20%; subtracting background levels did not reduce the discrepancy (Figure S2B). On the other hand, the qLiNE algorithm reported consistent intensity values (Figure 2C) with...
CV < 8% across different illumination conditions. The qLiNE algorithm was also robust to camera alignment relative to the reference card. We placed LFA strips on the reference card and took photos in different camera angles (roll, pitch, and yaw) as well as at varying distances (Figure 2D). For a given AuNP concentration, the qLiNE produced intensity values that were statistically identical regardless of the camera location.

**qLiNE Assay for CTS Detection.** We next established the protocol for the qLiNE assay. We adopted a competitive immunoassay scheme; because CTS is a small molecule (360 Da), it was difficult to find a pair of antibodies recognizing different epitopes.29,30 We synthesized CTS attached to BSA (CTSBSA) as a competitor against CTS in samples. To make a qLiNE-LFA test strip, we spotted CTSBSA on the test line of an NC membrane and anti-mouse IgG antibody on the test line (Figure 3A). The assay started by mixing a sample with anti-CTS Ab@AuNPs and wetting the end of the LFA test strip with the mixture (see Materials and Methods). Ab@AuNPs would capture CTS if present in the sample, and the CTS-Ab@AuNP complexes would then pass the CTSBSA at the test line to be captured by the anti-mouse IgG antibody at the control line. In the absence of CTS in the sample, Ab@AuNPs would be anchored on the test line by capturing CTSBSA. To maximize the assay sensitivity, we performed a series of comparison studies. We compared different anti-CTS antibodies for their compatibility with LFA (Figure S3). We also determined the optimal values (Figure S4) for (i) the composition of surfactant in the qLiNE-LFA working buffer; (ii) the CTS/BSA stoichiometry in CTSBSA synthesis; (iii) the amount of CTSBSA on an LFA strip; (iv) the LFA wetting time with a sample, and (v) Ab@AuNP concentration for the reaction with CTS in saliva.

**Figure 3A** shows photos of qLiNE-LFA strips taken under different illuminations (i.e., natural sunlight, no light, fluorescent light, and yellow light). At low CTS concentration, the optical signal was dominant at the test line. As the CTS concentration increased, the test line signal faded and the signal on the control line became stronger. Inspecting raw images permitted binary (yes/no) decision on CTS presence, but intensity values varied considerably per illumination (Figure S5A) and taking the ratio of raw intensities (= control/test) failed to compensate for the discrepancy (Figure S5B). Applying the qLiNE algorithm, however, made signal levels consistent regardless of the illumination condition (Figure 3B). We used qLiNE-adjusted intensity values, $S_T$ from the test and $S_C$ from the control lines, and defined a single CTS metric $Q_{CTS} = S_C(S_T + S_C)^{-1}$. Using this metric allowed for interassay comparison (Figure 3C), no statistical difference in $Q_{CTS}$ was observed among different illumination conditions ($P = 0.86$, two-way ANOVA; Figure 3C). We further used a different phone model and measured the same set of qLiNE-LFA strips (Figure S6A). The qLiNE app
installed in the phone analyzed images and produced QCTS values (Figure S6B). The results from these two different phone models were statistics identical (Figure 3D), which demonstrated high precision of the qLiNE approach. **Characterization of the CTS Assay.** We next characterized the overall performance of the qLiNE assay. To assess the sensitivity, we prepared samples by spiking varying amounts of CTS in buffer and saliva. Samples were processed by qLiNE-LFA to obtain QCTS values (Figure 4A). The assay results were robust against the media condition; titration curves were similar between buffer- and saliva-based samples. The dynamic range spanned 3 orders of magnitude, and the limits of detection were 0.17 (buffer) and 0.16 ng/mL (saliva), and the dynamical range was about 3 orders of magnitude. Data are displayed as mean ± s.d from duplicate measurements. Error bars are too small to be visible. (B) Comparison with ELISA. CTS-spiked saliva samples were analyzed by qLiNE-LFA and CTS-competitive ELISA. Data from both methods showed a good match. Data are displayed as mean ± s.d from duplicate measurements. (C) Selectivity test. Confounding biochemicals typically present in saliva had a negligible effect on the CTS signal even at their high concentrations (100 ng/mL). For a given CTS concentration (0 or 100 ng/mL), these biochemicals alone produced negligible signal, close to the assay background level (Figure 4C, left). Strong qLiNE signals were recovered when CTS was added into samples (Figure 4C, right).

We further evaluated the potential matrix effect caused by food consumption (Figure S7). As a representative example, we used coffee, chocolate, cola, orange juice, and milk. False-positive signals appeared on the test line when saliva contained these matrices at a high dose (10% by volume), but the signal dropped close to the background level at a lower dose (2% by volume). Based on this information, we recommended mouth rinsing with water before saliva collection.

**Stress Monitoring with Human Saliva.** Last, we applied the qLiNE assay for routine CTS monitoring (Figure 5A). The results were similar whether CTS was spiked in buffer or saliva. The limits of detection were 0.17 (buffer) and 0.16 ng/mL (saliva), and the dynamical range was about 3 orders of magnitude. Data are displayed as mean ± s.d from duplicate measurements. Error bars are too small to be visible. (B) Comparison with ELISA. CTS-spiked saliva samples were analyzed by qLiNE-LFA and CTS-competitive ELISA. Data from both methods showed a good match. Data are displayed as mean ± s.d from duplicate measurements. (C) Selectivity test. Confounding biochemicals typically present in saliva had a negligible effect on the CTS signal even at their high concentrations (100 ng/mL). For a given CTS concentration (0 or 100 ng/mL), these biochemicals alone produced negligible signal, close to the assay background level (Figure 4C, left). Strong qLiNE signals were recovered when CTS was added into samples (Figure 4C, right).

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normal CTS level is known to display a diurnal rhythm: it rises sharply with awakening from sleep in the morning, gradually declines during the day, and reaches the lowest point in the early morning hours during sleep. This pattern can be perturbed in response to external or internal stimuli, such as exercise, mental stress, and infection; monitoring CTS levels thus can inform person’s physiological homeostasis and help diagnosing hormonal disorders (e.g., Cushing’s syndrome and Addison’s disease).36,37

We first followed the charge of salivary CTS concentration during the day. Saliva samples were collected at four time points (7 am, 12 pm, 6 pm, and 11 pm) from six individuals and processed by qLiNE-LFA. For all six samples, CTS concentration was the highest in the morning and then decreased in the daytime into evening. The concentration continued to fall in four individuals who self-described themselves having no sleep disturbances (Figure 5B, left). For two individuals who self-described having chronic difficulty in falling asleep, the late-night (11 pm) CTS concentration was higher than that in the evening (6 pm) (Figure 5B, right). Note that elevated CTS levels before sleep were reported in insomniac patients.38

We next assessed whether qLiNE can detect CTS changes arising from daily activities. As an external stimulus, we chose exercise (>30 min in the afternoon). Saliva samples from nine individuals were collected before and after aerobic or resistance workout. We observed significant changes (P = 0.048; paired, two-sided t-test) in CTS concentration (Figure 5C). On average, CTS concentration increased (67%) after exercise sessions. We also checked how acute mental stress affects CTS levels. The chosen stressor was a public oral presentation. From 12 speakers, we collected a pair of saliva samples, one at regular night and the other a night before the presentation. CTS levels were elevated in 10 speakers before the presentation (Figure 5D), and the trend of CTS increase was statistically significant (P = 0.014; paired, two-sided t-test).

CONCLUSIONS

The qLINE approach could facilitate robust, quantitative biosensing at point-of-care and point-of-use settings. The key innovation was the combined use of a reference card and a software-based image processing; this approach automatically corrected imaging aberrations (e.g., illumination and alignment) and camera-specific variations, thereby extracting optical signals at high precision. Implementing qLiNE required no extra hardware, allowing most smartphones to be converted into a universal optical detector. Prior assay systems have also used reference cards for robust colorimetric analyses,39,40 but they needed recalibration for different phone types or ambient conditions.39,40 or performed regression analyses to correct the color space at each measurement.41 In contrast, recalibration was unnecessary and the color correction was much simpler with qLiNE. We proved the qLINE concept by optimizing it for CTS detection with LFA. When challenged under different ambient light settings, qLINE produced consistent analytical signal to enable unbiased comparison between assay results. The qLiNE-LFA also quantified CTS amount in saliva, which made it easy to detect CTS level changes induced either by biological rhythms or by stressors. End-users could perform this quantitative assay without the need to generate a calibration curve; sensor manufacturers can provide the most relevant calibration data through a remotely update in the qLINE app. We identified several technical aspects for future exploration. First, we may consider applying a deep neural network to improve the image correction algorithm.42 Using the smartphone-based qLiNE can speed up this process by allowing us to crowdsource a large number of images. Training the neural network with images from diverse use cases would make it more robust than the current algorithm. Second, we need to assess qLiNE performance with different color signals that can be generated by changing the size, shape, and the composition of nanoparticles.43 This study will test the robustness and universality of the qLINE algorithm. We should also consider adopting new blocking strategies,44,45 which would reduce nonspecific target binding on nanoparticles and enhance the assay specificity. Third, we can expand to other detection targets that require accurate quantification in colorimetric assays, including drugs, environmental hormones, food toxins, and proteins.42,46–48 With these improvements, qLiNE would make ubiquitous smartphones a power tool for various sensing applications, including personal healthcare, environment monitoring, and public safety.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c00793.

Signal analysis process of qLiNE platform; LFA signals analyzed by ImageJ; antibody screening for qLiNE-LFA; optimization test for the best performance in LFA; raw color intensities under different illumination conditions; qLiNE-LFA using a different phone model; matrix effect test; and comparison of point-of-use methods for CTS detection (PDF)

qLINE app operation (MP4)

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