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Arduino-Based Sensing Platform for Rapid, Low-Cost, and High Sensitivity Detection and Quantification of Analytes in Fluidic Samples †

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Abstract: Lateral flow assays (LFAs; aka. Rapid Tests) are inexpensive paper-based devices for rapid and specific detection of analyte of interest (e.g., COVID virus) in fluidic samples. Areas of application of LFAs cover a broad spectrum, spanning from agriculture to food/water safety, to point-of-care medical testing, and most recently, to detection of COVID-19 infection. While these low-cost and rapid tests are specific to the target analyte, their sensitivity and limit of detection are far inferior to their laboratory-based counterparts. In addition, rapid tests normally cannot quantify the concentration of target analyte and only provide qualitative/binary detection. We have developed a low-cost, end-user sensing platform that significantly improves the sensitivity of rapid tests. The developed platform is based on Arduino and utilizes low-cost far infrared, single-element detectors to offer sensitive and semi-quantitative results from commercially available rapid tests. The sensing paradigm integrated to the low-cost device is based on radiometric detection of photothermal responses of rapid tests in the frequency-domain when exposed to modulated laser excitation. As a proof of principle, we studied commercially available rapid tests for detection of THC (the principal psychoactive constituent of cannabis) in oral fluid with different concentrations of control positive solutions and subsequently interpret them with the developed sensor. Results suggest that the developed end-user sensor is not only able to improve the detection limit of the rapid test by approximately an order of magnitude from 25 ng/mL to 5 ng/mL, but also offers the ability to obtain semi-quantitative insight into concentration of THC in the fluidic samples.

Keywords: THC; lateral flow immunoassay; Arduino; point-of-need; photo-thermal radiometry

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1. Introduction

Lateral flow assays (LFAs; aka. Rapid Tests) are inexpensive paper-based devices for rapid and specific detection of analyte of interest (e.g., COVID virus) in fluidic samples. LFA tests can be performed on-site by non-specialized users, and are significantly cheaper than laboratory-based alternatives [1]. Because of these benefits, LFA tests are used in a wide range of applications, including agriculture [2], water safety [3], and point-of-care medical testing [4], including most recently, COVID-19 testing [5]. Consistent with widespread use of rapid tests, the global market of LFA industry is projected to reach a value of $12.6 billion USD by the year 2026 [6].

Despite obvious advantages of LFAs, these low-cost and rapid tests suffer from key limitations such as having far inferior limit of detection and sensitivity compared to standard laboratory tests. For example, in roadside testing for levels of THC (the principal psychoactive compound found in cannabis) in oral fluids, the limit-of-detection of commercialized LFAs are between 25–40 ng/mL, while the recommended legal *per se* limit is

between 0–2 ng/mL among different countries [7]. Similar situation holds for workplace safety screening of THC where the desired limits of detection of 4–10 ng/mL cannot be accommodated by existing LFAs. As such, determination of THC intoxication at roadside or workplace, currently, requires use of more invasive, time-consuming, and costly tests such as a blood test administered by specialized professionals.

There have been many efforts recently to retain the universal use and inexpensive attributes of the LFAs but increase their sensitivity and limit-of-detection. One such method proposed using LFAs containing fluorescent polymetric beads [8] as well as gold nanoparticles (GNPs), allowing for fluorescent interrogation of the LFA for enhancing the limit of detection. The drawback to this method is that standard LFAs are not currently manufactured using such fluorescent beads, and thus significant changes would have to be made in their manufacturing, which would likely increase their cost.

An alternate method which requires no change in LFA manufacturing is photothermal radiometry (PTR), wherein a focused laser light is used to excite the gold nanoparticles in a standard LFA, and the resulting thermal radiation is measured using an infrared camera [9]. Our recent works show that this sensing method can increase the sensitivity and limit of detection of LFAs by over an order of magnitude [10] while retaining the low-cost and rapid attributes of LFAs. A drawback with this method however is that it currently utilizes a thermal camera, which is prohibitively expensive for end-users, especially when LFAs are so highly valued for being inexpensive.

The purpose of this capstone project was to extend our recent innovations by integrating the sensing paradigm of photothermal radiometry in an Arduino-based system utilizing an inexpensive single-element far-infrared thermal sensor. Our results suggest that the significantly lower cost, end-user lab prototype significantly increases the sensitivity and limit of detection of LFA tests. As such, current work is expected to open the door for translation of our patented photothermal radiometry sensing platform to market as an end-user solution for significantly enhancing the detection performance of commercially-available rapid tests.

2. Methods

2.1. LFA Principles

An LFA test is performed by depositing a controlled volume of fluidic specimen onto the sample pad. This fluid is then pulled by capillary action through the membrane to the conjugate release pad, which contains the antibodies specific to the type of analyte being tested for. In a competitive-style LFA test, such as the oral-fluid THC test used in this study, the target analyte blocks the binding sites in the test line, allowing the antibodies to pass the test line and instead bind to the control line [11]. In such an arrangement, spiking the LFA with specimens containing a concentration of analyte above the detection limit of the test (aka. a positive outcome), yields a test strip with non-existent test line and a visible control line. This can be seen in Figure 1.

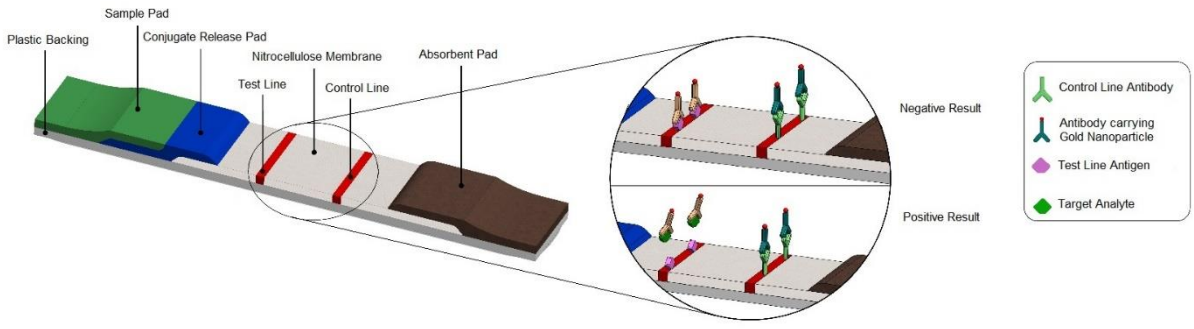

Figure 1. A competitive style LFA where the presence or absence of target analyte either blocks or allows binding of antibodies to the antigens in the test line.

2.2. LFA Sensing via Photothermal Radiometry (PTR)

LFAs use GNPs as visible indicators in both the test and control lines. These GNPs selectively reflect ambient lighting, leading to colorimetric signals (normally dark purplish color) at test and control lines. The innovative PTR sensing method, however, takes advantage of selective absorption spectra of GNPs, meaning there are specific wavelengths at which light is absorbed very well by these GNPs but very weakly by the surrounding nitrocellulose paper [12]. Accordingly, the PTR sensing method excites LFAs with a laser of specific wavelength to induce amplified thermal responses. Such responses can simultaneously be measured with infrared (IR) sensors and then quantified to yield signals that are proportional to the extent of accumulation of GNP-analyte pairs at LFA lines. To make PTR sensing method insensitive to varying ambient temperatures, or other sources of ambient thermal noise, the utilized laser excitation is intensity modulated at a specific frequency (e.g., 1 Hz), creating thermal wave fields within the thickness of LFAs. Demodulation of acquired IR signals at the specific frequency enables removal of environmental thermal effects to enable very sensitive detection and quantification of light absorption signatures of the GNPs [13].

2.3. Sensor Design Process and Final Design

The purpose of this capstone project was to demonstrate the feasibility of using inexpensive single-element IR sensors to produce results that were comparable to those produced by a thermal-camera based system. To achieve this goal, an initial design was proposed which utilized an inexpensive single-element far-infrared sensor ($9; Wavgat Store, China) and 0.6 W laser diode ($20; 808-nm; Besram Technology Inc, Wuhan, China). Then, a parametric optimization design process was pursued. That is, one of the design variables (e.g., laser beam size) was changed, tests were performed on the control LFAs, the configuration performance was compared with previous configurations, and further configuration changes were then recommended until the design parameters were optimized.

The final optimized design composed of a hinged box with electronic components, consisting of Arduino-base controller, laser driver, servo driver, and power supply, housed below the testing deck. The device and system arrangement can be seen in Figure 2. The testing deck holds a rack-and-pinion mechanism with a slot for insertion of an LFA cassette. This mechanism allowed for the LFA to be automatically moved so that the same laser-sensor pair could be used to interrogate both the test and control line. The laser was fixed at an optimal angle of incidence and distance to the LFA and controlled by the laser driver. The single-element far-infrared sensor mounted perpendicular to the line under test and was sampled by the Arduino-based controller.

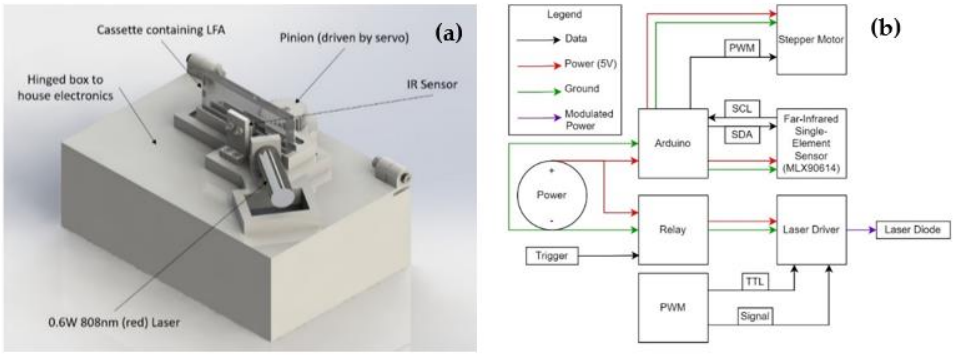

Figure 2. (**a**) Rendering of the device (**b**) Electronic system overview featuring the laser and motor control systems, and sensor sampling scheme.

2.4. Data Processing

During a test, datasets are collected separately for the test and control lines of an LFA. Both datasets contain a time series of radiometric measurements from the single-element sensor. Because of hardware limitations, the time series do not have perfectly spaced samples in time, so the series were then interpolated and resampled at regular time intervals to enable frequency-domain analysis of the signals. A Fast Fourier Transform (FFT) was then performed on the resampled time-series to transform the dataset to the frequency domain. The intensity of each response was then determined by comparing the response at the laser modulation frequency (1 Hz) to the respective noise floor. Analysis of the signals in the frequency-domain via the FFT algorithm allows for effective rejection of signal noise which is specifically essential when the signal-to-noise ratio is poor (i.e., test line color is faint). Finally, the response of the test line was normalized to the response of the control line. This normalized response (aka. amplitude metric) was found to be strongly correlated to the concentration of analyte in the fluid under test in the corresponding LFA. Figure 3 shows representative interpolated and resamples time series and the corresponding frequency-domain spectra for an LFA's control and test lines. As expected, thermal-wave response is stronger for the control line in both time and frequency domains compared to those of the test line.

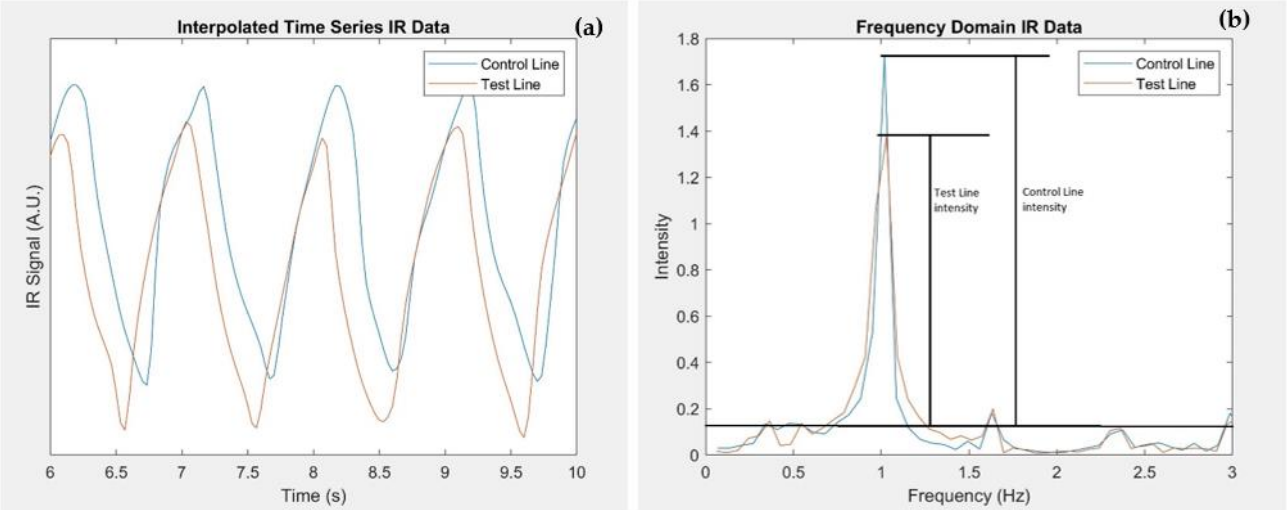

Figure 3. (**a**) Interpolated and resamples time series of control and test line responses (**b**) Control and test line responses transformed to the frequency domain.

2.5. LFA Preparation/Samples

To test the performance of developed system, ten LFAs were spiked with five known concentrations of THC (i.e., two spiked LFAs at each of the five concentrations). Spiking solutions were prepared by mixing a known volume of Delta-9 THC stock solution (Sigma Aldrich; Oakville, ON, Canada) with known volume of artificial saliva (Pickering Laboratories Inc.; Mountain View, CA, USA). Measured quantities of this solution were then serially diluted to obtain THC concentrations of 0 ng/mL, 2.5 ng/mL, 5 ng/mL, 10 ng/mL, 25 ng/mL. Ten LFAs (NarcoCheck; Montluçon, France), each with a cutoff concentration of 25 ng/mL, were then spiked using 200 uL of their respective solution and labelled accordingly.

3. Results and Discussion

Figure 4 depicts the mean ± STD amplitude metric data collected from the ten LFAs, each sampled ten times. Given the competitive style design of LFAs, as THC concentration increases the test line fades out, leading to smaller amplitude metric signal. Results

depicted in Figure 4a show that for THC concentrations greater than 2.5 ng/mL, the amplitude metric of test line is significantly smaller than that of the LFA's control line. As such, the limit of detection of developed end-user prototype was measured at 5 ng/mL.

Figure 4b shows the collected data from all THC concentrations tested. Qualitative assessment of the plot shows correlation of end-user output with THC concentration. To assess the data quantitatively, one-way ANOVA analysis of means was performed which suggested presence of statistical significance ($p < 0.05$). As such, the Tukey HSD post-hoc pairwise comparison was conducted to identify which pairs of means were significantly different. As depicted in Figure 4b, results suggest that end-user device can reliably resolved the THC concentration of the sample into one of four categories (<5 ng/mL, 5–10 ng/mL, 10–25 ng/mL, >25 ng/mL). Results of Figure 4 show two major improvements: (1) the end user device enhanced the detection limit from the nominal LFA threshold of 25 ng/mL to 5 ng/mL; (2) the end user device enabled semi-quantitative detection of THC into 4 concentration categories (most of which are below nominal detection threshold) as opposed to the conventional binary categorization of the LFA (i.e., <25 ng/mL OR >25 ng/mL). We would also like to note that more tests with larger sample sizes is required to further confirm the findings of this feasibility study.

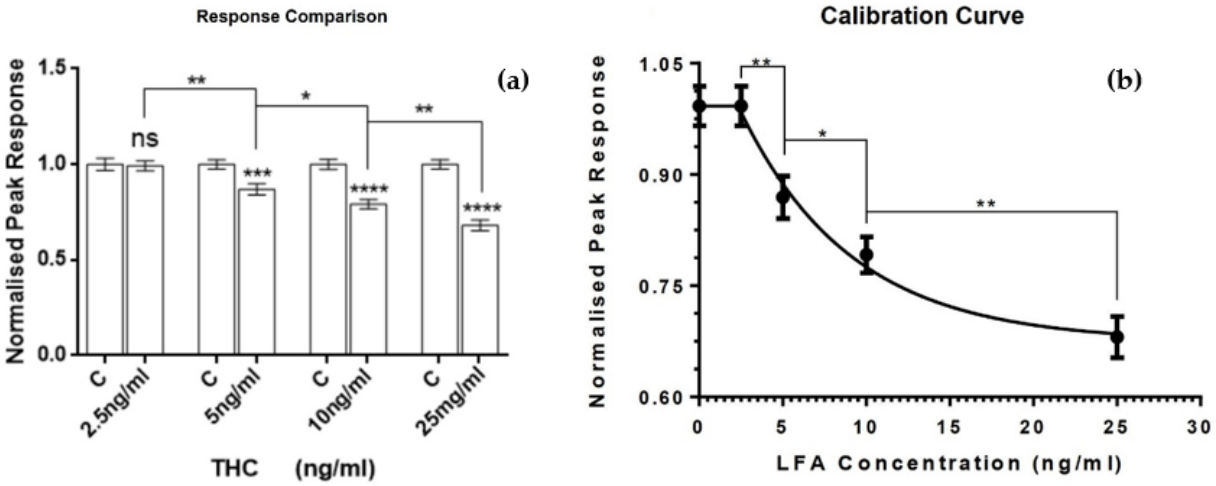

Figure 4. Results of 10 repetitions per LFA (N = 20 per concentration) (**a**) Control line versus normalized test line response (**b**) Normalized responses fit to a quadratic curve.

Hardware costs for this prototype are found in Table 1. This device is considerably less expensive than even the cheapest thermal camera-based LFA reader, which is about USD $350 [10]. With further development and optimization for mass manufacturing, the current USD $51 cost using off-the-shelf components in expected to decrease significantly further to further facilitate the translation of our innovation to the market as an end-user device.

Table 1. Summary of hardware costs for the prototype model.

Component Category	Cost (USD)
Laser + electronics	$ 20
IR Sensor	$ 9
Arduino-based controller	$ 15
Housing and motor	$ 7
Total	$ 51

This capstone project served as a feasibility study, thus there is still much room for performance improvement. One possibility would be using a laser of a different

wavelength at which GNPs are more absorptive. The current sliding mechanism is also prone to failure and a new method should be used to interrogate both lines simultaneously. More LFAs should also be prepared at a variety of different concentrations and at larger sample sizes for robust validation of end-user system performance.

4. Conclusions

In conclusion, under the scope of an undergraduate capstone project, we designed and developed a low-cost and end-user laboratory prototype of a sensing device for enhancing the detection performance of rapid tests/LFAs. Developed end-user device utilizes an intensity-modulated laser source to excite LFA GNPs while registering their thermal-wave responses with a single element infrared sensor. Frequency-domain processing of radiometric signals enable sensitive detection and quantification of photothermal radiometric responses of LFAs which translated to significant enhancement of LFA nominal detection threshold. Our results also show that through calibration the end user device can coarsely quantify the concentration of analyte used for spiking the LFA. With further development, the performance and robustness of this device is expected to be improved while further reducing the cost of system.

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