Handheld Thermo-Photonic Device for Rapid, Low-Cost, and On-Site Detection and Quantification of Anti-SARS-CoV-2 Antibody

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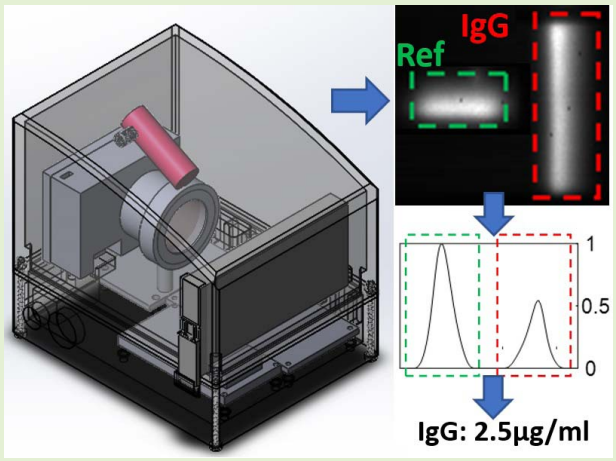
***Abstract*—With the emergence of vaccines and antibody therapeutics, rapid and scalable platforms are needed to quantify the antibody response of individuals. Lateral flow immunoassay (LFA) based test strips provide a rapid, low-cost, and point-of-care approach to antibody testing against the SARS-CoV-2 virus. These convenient and scalable tests, however, are qualitative in nature and cannot quantify the immune response of the infected and/or vaccinated individuals. This study reports on the development of a rapid, low cost and portable thermo-photonic device that enables sensitive detection and quantification of antibody levels using commercially available COVID-19 Antibody LFAs. Unlike conventional LFA readers, the developed technology is based on sensing the infrared thermal radiation of tag gold nanoparticles following laser excitation (aka photothermal response). Our proof-of-concept results with humanized monoclonal anti-SARS-CoV-2 Spike receptor-binding domain (RBD) IgG demonstrate that the thermo-photonic technology can detect and quantify antibody concentrations within the clinically relevant range and with a limit of detection of 0.1 μg/ml. The reader in conjunction with antibody LFAs offers a low-cost, portable, and scalable solution for assessment of the degree of immunity in populations, quality control of convalescent plasma donations for antibody therapeutics, and monitoring the immune response of infected individuals and vaccine recipients.**



***Index Terms*—Coronavirus, COVID-19, active thermography, lock-in-thermography, vaccine, SARS-CoV-2, antibody.**

I. Introduction

THE COVID-19 pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to a dramatic loss of human life worldwide, particularly in the elderly populations and for persons with underlying medical conditions. As of May 6, 2021, more than 155 million coronavirus cases have been reported and over 3.2 million people have died [1]. At the onset of the pandemic, the primary focus of countries was to rapidly identify the infected individuals and isolate them from others to prevent the spread of COVID-19 [2]. With the emergence of vaccines and antibody therapeutics, however, the need and importance of antibody detection and quantification technologies have significantly increased. SARS-CoV-2 infections typically elicit neutralizing antibody responses [3], [4]; an analogous pattern of immune response occurs after the administration of vaccines [5]–[8]. However, how long immunity produced by vaccination will last is not known yet. Comprehensive longitudinal data from the vaccinated populations is needed to know how well the vaccines work in the long run, especially with the frequent discovery of new coronavirus variants [9]. Quantitative antibody tests have the promise to offer means for collecting such longitudinal data and/or screening the immunity of individuals overtime at population and end-user levels [10]. Antibody therapeutics is another area that can benefit from the development of quantitative antibody tests. Transfer of convalescent plasma appears to offer clinical benefit for severe but not critically ill patients [11] and is approved by the United States Food and Drug Administration (FDA) under the emergency investigational new drug category [12], [13]. Quantitative analysis and quality control of donor's antibody level is, therefore, essential for the selection of convalescent

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plasma donors for the treatment of COVID-19 patients [14], especially since recent studies show the rapid decline of Immunoglgeobulin G (IgG) and neutralizing antibody levels with time [15], [16].

At present, neutralization assay [17], chemiluminescent assay (CLIA) [18], enzyme-linked immunosorbent assay (ELISA) [19], [20] and lateral flow immunoassay (LFA) [14], [21] are the most common tools for assessing the antibody response against SARS-CoV-2. Most of these methods are either qualitative or semi-quantitative in design and focus on the detection of the presence of antibodies above a certain threshold rather than the quantification of their titers. The neutralization assay, which remains a gold standard method for determining antibody efficacy, is a lab-based test that uses live SARS-CoV-2 virus to determine if patients' antibodies can prevent viral infections in a specialized biosafety level 3 containment facility [22], [23]. Consequently, the neutralization test method is tedious, time-consuming, and not deemed suitable for large-scale serodiagnosis and vaccine evaluation [4]. CLIA is an immunoassay technique based on luminescence. It has the advantages of being reliable, fast, and technically simple; however, the inevitable high cost of CLIA prohibits its scalability [18]. ELISA is a popular lab-based method that can quantify the antibody levels through calibration but normally involves a long assay time ($>$ 2 hours), requires the involvement of skilled personnel, and also the use of expensive instruments and consumables [7]. The LFAs, on the other hand, are low-cost paper-based test strips widely used in medicine for point-of-care screening and diagnostics. While LFAs offer convenient, low-cost, and rapid detection of COVID-19 antibodies in human blood, serum, or plasma, the test results are only binary (positive/negative) and cannot be readily used for the quantitative assessment of a patient's anti-SARS-CoV-2 antibody levels [7].

The above brief review of the current technology landscape suggests that there is currently an unmet need for COVID-19 antibody quantification technologies that are not only accurate but also rapid, portable, low-cost, and scalable. To address this need, we have recently developed and patented [24] a bench-top thermo-photonic reader technology for sensitive interpretation of LFAs. Our recent publications demonstrate that thermo-photonic interpretation of LFAs not only improves the accuracy and the limit of detection of LFAs but also allows for accurate quantification of a target analyte [25]–[28]. The major limitation of our previous works is that they were all carried out with benchtop systems utilizing either expensive infrared cameras or bulky laser systems. Here in this work, we report on the development of a low-cost, portable handheld thermo-photonic device for the quantification of target analyte in LFAs. To demonstrate the efficacy of the low-cost handheld device, we present and discuss our experimental results on the detection and quantification of anti-SARS-CoV-2 IgG antibodies in commercially available low-cost COVID-19 IgG/IgM LFA test cassettes. Our study on the humanized monoclonal anti-SARS-CoV-2 IgG antibody shows that the thermo-photonic technology in conjunction with COVID-19 IgG/IgM LFAs facilitates rapid, and quantitative analysis of antibodies, offering a low-cost, portable and scalable solution for assessment of the immune response of COVID-19 patients and vaccine recipients.

II. Methods

A. Humanized Antibody

Humanized monoclonal anti-SARS-CoV-2 Spike receptor-binding domain (RBD) IgG solution (MBS355896) from MyBioSource Inc. (San Diego, USA) was used in this study as the positive control. The solution was supplied in 0.01M of Phosphate Buffered Saline (PBS) with an IgG concentration of 10 μg/ml. The specimen was stored at -20°C and brought to room temperature before use. Nine different concentrations of IgG (10 μg/ml, 5 μg/ml, 2.5 μg/ml, 1.25 μg/ml, 0.8 μg/ml, 0.5 μg/ml, 0.2 μg/ml, 0.1 μg/ml, 0 μg/ml) were prepared by serial dilution of the stock solution with 0.01M PBS (MilliporeSigma Canada, Co., Oakville, Canada).

B. COVID-19 Antibody Cassettes

Commercially available immunochromatographic test cassettes from BTNX Inc. ($8; Rapid Response^{TM} COVID-19 IgG/IgM Rapid Test Device, Markham Ontario) were used in this study. These qualitative LFA strips are designed with the nucleocapsid and RBD antigens to detect SARS-CoV-2 IgG and IgM antibodies. In this feasibility study, we only examined the capability of our portable technology (Fig. 1a) in detecting and quantifying the IgG antibody as IgG is believed to be the dominant antibody providing long-term immunity [29]. The principle of operation of these LFAs is as follows: the sample containing the analyte (e.g., IgG) is applied to the sample well of the LFA. The sample flows along the strip due to the capillary action and the target analyte binds with the antigen-gold nanoparticle (GNP) conjugates at the conjugate pad. The antigen-GNP-analyte complexes further flow in the paper device and eventually accumulate on the test line to create a visual contrast proportional to the concentration of analyte in the sample. Similarly, antigen-GNP conjugates accumulate on the control line and create visual contrast. Therefore, a positive SARS-CoV-2 IgG test shows two colored lines: a dark purple test line for IgG, and a red control line. While the presence of a control line suggests a properly spiked test, the color intensity of the test line offers a direct correlation with the concentration of the IgG in the sample. The source of these colorimetric signals is the extinction of the light from the surface of colloidal gold nanoparticles (GNPs) immobilized on the LFAs' test line [30]. That is a higher concentration of IgG results in immobilization of a larger number of GNPs on the test line, producing a stronger colorimetric signal.

To test the efficacy of thermo-photonic sensing in quantifying IgG concentration, BTNX Inc. assays were spiked with test solutions containing various concentrations of IgG. Spiking of assays was carried out according to the manufacturer's guidelines. That is, 5 μl of the test solution was dispensed in the sample well, followed by the addition of two drops of assay dilution buffer (approximately 50 μl) to drive the capillary action along the strip. Per manufacturer instruction, LFA strips were interpreted 15 minutes after spiking.

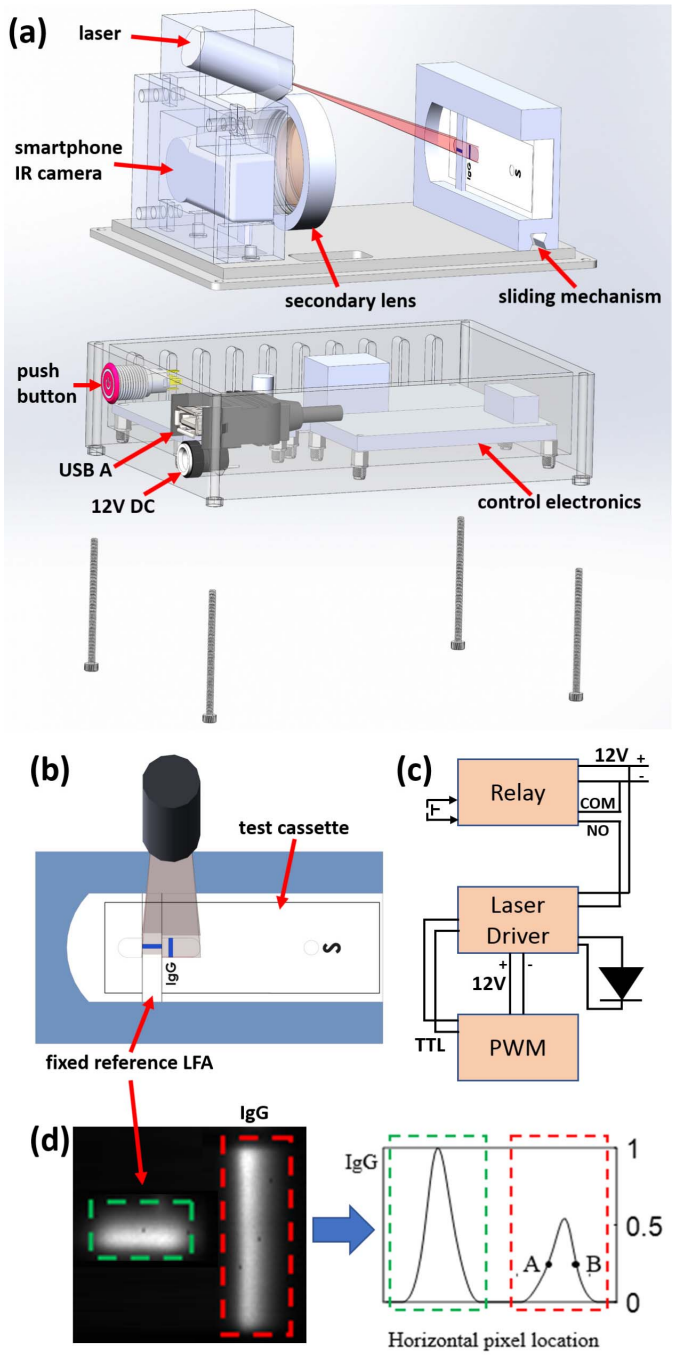

Fig. 1. (a) Exploded view of developed low-cost and portable thermo-photonic lock-in thermography device. (b) Schematic representation of laser beam orientation with respect to test cassette and the fixed reference LFA spiked with 10 μg/ml of IgG (used for normalization). (c) A low-cost and compact electronic control circuit. (d) LIT Amplitude image of a cassette spiked with 2.5 μg/ml of IgG (red box) next to the reference LFA (green box). Averaging the boxes along their long axis leads to two bell-shaped curves. The maxima of the reference curve is used for normalization while the average of amplitude values within the full width at half maximum points of the test curve (A and B) is adopted as an amplitude metric used in the study.

To examine the reproducibility of results, eight test cassettes were spiked at each IgG concentration. Moreover, each assay was interpreted five times with the thermo-photonic reader to examine the repeatability of results. After data collection, the thermo-photonic signal strengths of the assays were statistically compared to explore the possibility of quantifying IgG concentrations in sample solutions.

C. Portable and Low-Cost Thermo-Photonic Lock-in Device for Interpretation of COVID-19 Antibody Cassettes

Lock-in thermography (LIT) is an active thermo-photonic method that is frequently used in industry for the detection of defects in manufactured parts [31]. In such applications, the subsurface defects and inhomogeneities effectively act as thermal impedance and result in a larger amplitude of thermal wave of the defective regions compared to those from the surrounding intact areas. A similar principle applies to the LIT measurements of LFAs; here, the intensity-modulated 808nm laser excitation is selectively absorbed by the GNPs immobilized on the test line due to surface plasmon resonance. Since the number of GNPs at the test line is proportional to the concentration of the target analyte (e.g., IgG), the amplitude of the measured LIT signals/images can be used for the quantification of the analyte concentration. Accordingly, in our experiments, after spiking the SARS-CoV-2 antibody test strips with different concentrations of the humanized IgG antibody, the LFA strips were interrogated by a low-cost, handheld, and portable LIT device (Fig. 1a).

The device hardware is composed of four major components: excitation, detection, electronics, and mechanical housing sub-systems. Excitation of LFAs was carried out with a low-cost 808nm laser diode that was installed in a cylindrical laser-lens housing ($20; Besram Technology Inc, Wuhan, China). It offers 600mW of optical power in a rectangular format as shown in Fig. 1b. Detection of infrared radiation from the LFAs was achieved with a low-cost smartphone infrared camera ($255; Seek Thermal Compact Android) in conjunction with a secondary low-cost Zinc Selenide lens ($10; MCWlaser Inc, Wuhan, China). For system electronics (Fig. 1c), a generic timer relay circuit ($2.5) was used to provide electric power to the system for 30 seconds upon push button triggering. The laser driver ($12; Wuhan Jingluyao Trading Co., China) provided intensity-modulated electric current to the laser diode based on the 1Hz TTL signal received from a generic PWM circuit ($1.5). Device hardware was housed in a stacked form in a 3D printed enclosure (Fig. 1a) which allows insertion of the COVID-19 antibody test cassette through a sliding mechanism. The overall size and cost of the handheld device are $8 \times 11 \times 10\text{cm}^3$ and ~US$350, respectively. Device software was developed in C# and LabVIEW. Our previously reported custom-made software development kit (SDK) was utilized for reliable control of smartphone IR camera and synchronized acquisition of infrared frames through USB 2.0 interface [26]. After data acquisition, lock-in demodulation of acquired infrared thermal signals was instantaneously carried out in the LabVIEW environment to obtain the thermo-photonic amplitude images of the LFA strips. The intensity values of LIT amplitude images represent the amount of modulated heat (aka thermal wave amplitude) measured across the sample surface.

The test cassette used in this study utilizes two different kinds/sizes of nanoparticle tags for the test and control lines,

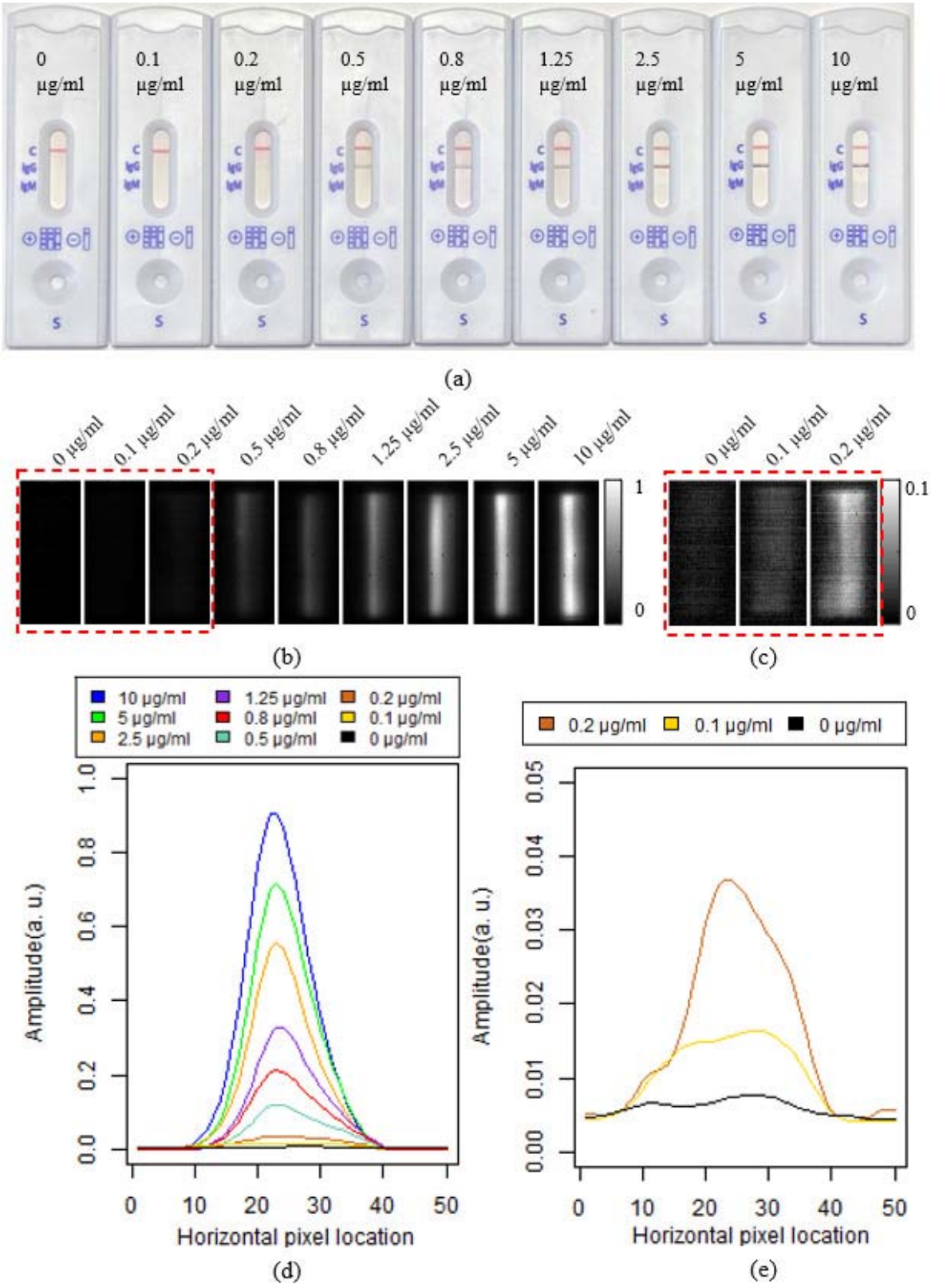

Fig. 2. (a) Representing photographs of COVID-19 IgG/IgM test cassettes developed at different concentrations of IgG. (b) Corresponding thermo-photonic amplitude images of LFAs in (a). (c) The thermo-photonic images of the three lower concentrations (0.2 μg/ml, 0.1 μg/ml, and 0 μg/ml) using a finer scaled colorbar. (d) Normalized LIT amplitude profiles of LFAs in (b). (e) Magnified view of the normalized LIT amplitude profile of the three lower concentrations, highlighting the ability of LIT in distinguishing between very small concentrations of IgG and in absence of a colorimetric signal.

as realized in Fig. 2a by their different colors. Consequently, these nanoparticles have different surface plasmon resonance and hence different optical properties (scattering and absorption peak wavelengths). While the dark purple-color test line efficiently absorbs the 808nm laser of our system, the absorption by the red-color control line is negligible. As such, signals from the control line could not be used as a reference to compensate for day-to-day variations of laser source power.

To overcome this limitation, the test line of an LFA spiked with 10μg/ml of IgG was cut and permanently placed in the field of view of the LIT device and always imaged together with the test LFA strip (Fig. 1b). The signal from this fixed reference test line was used for signal normalization. That is, after LIT imaging of each test strip, the amplitude image was scaled between the mean signals obtained from the reference test line (green rectangle in Fig. 1d) and those of the surrounding white nitrocellulose paper. This normalization step minimizes the systematic errors induced by day-to-day variations in laser illuminations and improves the repeatability of the LIT system. Subsequently, the average intensities of pixels in all columns of the test strip (red rectangle in Fig. 1d) and all rows of reference strip (green rectangle in Fig. 1d) were calculated to produce a profile with 2 bell-shaped curves representing the reference and sample test lines. For quantitative analysis, a metric was defined (aka. amplitude metric) by averaging the amplitude values within the full width at half maximum (FWHM) of the sample bell-shaped curve (between A and B in Fig. 1d). Our previous studies on the immunochromatographic test cassette for THC [27] show that the amplitude metric highly correlates with the concentration of analyte present in the solution and can be used for developing a calibration curve for the quantification of the analyte. Therefore, in this study, we adopted the same amplitude metric for the quantification of IgG in spiked LFAs.

D. Data Analysis

All the statistical analyses in this study are conducted using R™ (v3.6.3). The conventional one-way ANOVA has an assumption that all groups share a common variance. Therefore, the homogeneity of variances is tested by using Levene's test. If the test shows a significant result ($p<0.05$), then Welch's ANOVA is conducted instead of one-way ANOVA. If ANOVA shows a significant difference between the mean values, Tukey HSD post-hoc pairwise comparison is conducted to identify which pairs of means are significantly different. If the homogeneity of variance assumption is violated, a Games-Howell post hoc test is adopted instead of Tukey HSD.

III. Results

Figures 2a and 2b depict representative visual and LIT images of COVID-19 IgG/IgM test cassettes spiked with different concentrations of IgG. Although test lines are visible in visual photographs of all the IgG concentrations higher than 0.2μg/ml, a rapid decline of color intensities with a decrease in IgG concentration makes it unreliable to visually distinguish between samples with comparable IgG concentrations (e.g., 2.5μg/ml vs 5μg/ml vs 10μg/ml or 0.5μg/ml vs 0.8μg/ml). Thermo-Photonic LIT images of the panel (b), however, exhibit a continuous change of contrast with IgG concentrations. The IgG test line at 0.1 and 0.2 μg/ml are barely visible (if visible at all) in the visual image (Fig. 2a) whereas they are clearly recognized in the thermo-photonic image with finer scaled colorbar (Fig. 2c), highlighting the intrinsic ability of LIT in sensitively sensing signals from even very small accumulations of GNPs. The normalized amplitude profiles of different IgG concentrations are depicted in Fig 2d. Qualitative correlation of LIT signals with IgG concentration and the ability to distinguish any two IgG concentrations can clearly be recognized. Figure 2e shows the normalized amplitude profile of three lower concentrations (0.2μg/ml, 0.1μg/ml, and 0μg/ml), suggesting the ability of the low-cost device in distinguishing between very small IgG concentrations. Note that visually, no colorimetric signal can be seen for these three lower concentrations, Fig. 2.a.

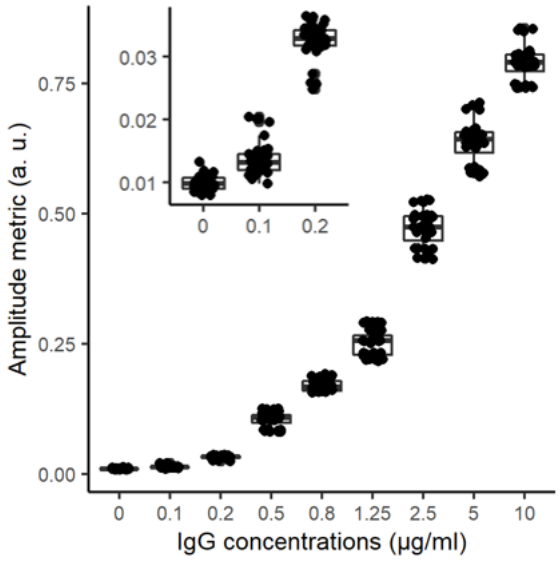

Fig. 3. Box plot with dots showing the entire distribution of data at each IgG concentration.

Box plot with dots of Fig. 3 shows the qualitative correlation of the IgG concentration with the amplitude metric. This plot displays the entire distribution of data within each IgG concentration; each column (group) displays the amplitude metrics of 40 measurements carried out at a given IgG concentration. The box plot shows no overlap in the distribution of data between any two concentration groups except between 0 μg/ml and 0.1 μg/ml. The embedded plot shows the magnified view of distribution in low concentration groups. Figure 4 shows the corresponding plots of the mean amplitude metrics of groups vs. the concentration of IgG on a logarithmic scale. The 95% confidence interval error bars between any two concentrations do not overlap, suggesting a significant difference between mean values. Consequently, the plot of Fig. 4 can serve as a calibration standard for quantifying the IgG titer in the serological analysis.

Both the boxplot (Fig. 3) and line plot (Fig. 4) qualitatively show unequal variances for different IgG concentration groups. To test the uniformity of variance, Levene's test was performed. Levene's test showed unequal variances

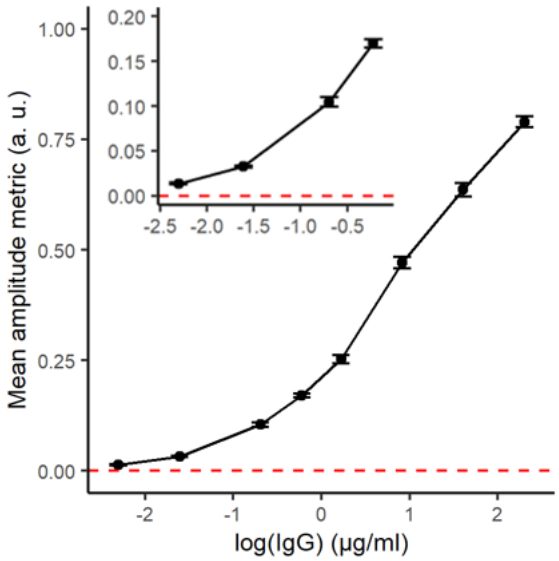

Fig. 4. Mean amplitude metrics at different concentrations of IgG in log scale with 99% confidence interval error bars.

($P<0.001$) between different IgG groups. Therefore, Welch's ANOVA was performed to compare mean amplitude metrics at different IgG concentrations. The means were significantly different among different concentration groups ($p < 0.01$). Games-Howell post-hoc test further confirmed that all the pairwise group comparisons were significantly different ($p < 0.01$). The statistical power of 1 and effect size of 0.99 was obtained at a significance level of 0.05 and a sample size of 40 in each concentration group. The coefficient of variation (CV) was also calculated to examine the repeatability of the measurements. The CV is normally calculated by taking the ratio of the standard deviation and the overall mean and is usually expressed as a percentage. The averaged CV obtained from all the test cassettes was 1.89%, demonstrating high repeatability of LIT measurements.

IV. Discussion and Conclusion

The novel coronavirus infection induces neutralizing antibodies, such as IgA, IgM, and IgG. The presence of IgM and IgA in the blood are normally seen as indicators of active or recent infection of SARS-CoV-2. While IgA and IgM antibodies decay fast over time, the IgG antibody that develops later following infection remains in the blood for a longer period [32]. Similar to IgA, IgG is associated with viral neutralization activity which is crucial for recovery from the infection [32]. Therefore, the concentration of IgG antibodies in the blood can be an accurate indicator for monitoring the immune response of convalescent patients over time [19]. This feasibility study demonstrates the efficacy of developed low-cost and portable thermo-photonic devices in rapid detection and quantification of SARS-CoV-2 IgG using the humanized antibody.

Recently, Peng et. al. [33] reported on a photon-counting technology for quantification of the IgG in LFAs. While the limit of detection achieved in this work is better than ones needed in the clinic (as well as that of ours), the photon-counting technology is a benchtop system containing bulky expensive equipment and parts with a mechanical motion that are not fit for scalable administration of technology to the points of need. Development of paper-based IgG ELISA kits for the detection of anti-SARS-CoV-2 antibodies has also been reported [34] using a CMOS camera and base on the intensity of visible-light scattering from immobilized tag particles. However, the reported detection limit does not satisfy the clinical need and much inferior to that of the developed thermo-photonic device (9 μg/ml vs 0.1μg/ml). The thermo-photonic technique operates based on selective absorption of light by GNPs and in absence of baseline/noise signals from the surrounding medium and as such is highly sensitive compared to light scattering-based readers. Moreover, the modulation of optical excitation in the Thermo-Photonic method results in the generation of diffusion thermal waves which thermally conduct and interrogate the entire thickness of the LFA test line. The enhanced detection sensitivity and specificity of Thermo-Photonic LIT sensing has also been shown in other fields such as early detection of dental caries [35]–[37] and detection and quantification of lipid in tissue [38]. To our knowledge, this is the first report on a low-cost and handheld solution that can rapidly quantify antibody titer against SARS-CoV-2 within the clinically relevant range.

The developed low-cost and portable reader technology has great potential to make impactful contributions to the management of the current pandemic. For example, the effectiveness of the plasma therapy for COVID-19 patients strongly depends on robust antibody response in convalescent plasma donors. However, a recent study shows that IgG and neutralizing antibody levels start to decline within 2-3 months after infection in a high proportion of individuals [15]. Ibarrondo et. al. [15] collected 70 samples from 34 patients with mild COVID-19 in 18-119 days duration after the onset of the symptoms. The IgG levels in these samples were 0.1μg/ml and higher in all measurements which indicates that the enhanced sensitivity and detection/quantification range of the Thermo-Photonic technology perfectly align with the range of IgG titers seen in the clinic. The developed device, therefore, can potentially be utilized in health care units to measure the antibody titer of the individuals with mild covid-19 in the early convalescent phase as the limit of detection of the reader for total RBD specific IgG titers is at least 0.1‘ μg/ml. The upper range of quantification in this study was limited by the concentration of IgG in the control positive solution (10 μg/ml). We do not anticipate any fundamental limitation in measuring very high concentrations of antibodies because samples can always be diluted with known volumes of neutral buffers to enable measurements within the detection range of sensors, as routinely done in ELISA.

The thermo-photonic reader also has the potential to be used for monitoring/screening the changes in antibody levels for evaluating treatment and prognosis. Studies found that individuals with low levels of anti-viral IgG are more likely to be seronegative [15], [16]. A low-cost and rapid technology, such as the developed portable reader enables a timely serosurvey to prioritize the administration of vaccines. The development and administration of mass vaccinations have raised hopes to individuals that the COVID-19 will be overcome soon.

As of May 6, 2021, fourteen vaccines have been authorized around the globe and sixty vaccines are in the development phase [39]. Many countries anticipate immunizing their target populations by the end of September 2021. However, it is not clearly known how long the immunity produced by a vaccination lasts and when is the optimal time to administer booster vaccine shots; more data and longitudinal seroprevalence studies are needed to fully understand the duration of immunity. Our technology can play a key role in longitudinal monitoring of the immune response of the vaccine recipients and hence enable evaluation of the vaccine efficacy at the population level. To test this possibility, we are currently in the process of validating the efficacy of the developed system with blood/serum from COVID positive patients at local hospitals and the bedside. Cross-reactivity and substance interference studies performed on the COVID-19 IgG/IgM test cassettes used in this study have demonstrated that the performance of the assay is not affected by antibodies of common human diseases or other substances normally present in the human serum/blood [40]. Therefore, we anticipate that the low-cost and portable device in conjunction with COVID-19 IgG/IgM test cassette offer an affordable solution for sensitive detection and quantification of COVID-19 antibodies at point-of-care.

The cost of the developed handheld device (∼US$350) is orders of magnitude smaller than either CLIA or ELISA. The measurement duration is less than 30 sec (excluding ∼15-minute LFA development time) which is significantly faster than ELISA that takes >2 hours. Therefore, the thermo-photonic reader in conjunction with low-cost ($8) colloidal GNP-based LFA can significantly reduce the time and cost of quantification of antibody titers, offering a promising scalable alternative to ELISA and CLIA. While this feasibility study was limited to the detection and quantification of IgG, our technology is a platform technology for sensitive and quantitative interpretation of any LFA utilizing GNPs. We anticipate our system to be able to be adapted to detect and quantify other types of antibodies such as IgA and IgM.

In this work, we employed an 808 nm laser diode to excite the nanoparticles. While better absorption cross-sections can be achieved at shorter wavelengths [30], [33], the low-cost aspect of high power 808nm laser diodes enables the manufacturing of a low-cost handheld solution suitable for scalable administration in the community for better management of the current pandemic. For example, the developed technology has great potential to be used as a point-of-care diagnostic device for rapid detection and quantification of antibodies against coronavirus to confirm SARS-CoV-2 immune cases and the efficacy of vaccines. A recent study shows that a single dose of mRNA vaccine in the individual with past infection elicits an immune response comparable to that of two doses of vaccination in individuals who have not been infected with SARS-CoV-2 in the past and suggested quantitative antibody testing prior to vaccination [41]. The developed thermo-photonic technology can enable such patient-specific vaccination policies. The effect of ambient temperature is not expected to have a significant effect on the performance of the developed thermo-photonic device because the device employs LIT technology that interrogates the temporal cyclic variation (aka modulation amplitude) of sample temperature at laser modulation frequency rather than the absolute temperature of the sample. The normalization process further minimizes the effect of the environmental variables as the test lines from the test and reference LFAs as well as the surrounding nitrocellulose paper are all exposed to the same environmental conditions.

In summary, we have developed a portable and low-cost thermo-photonic device for sensitive quantification of low-cost LFAs and tested its performance on COVID-19 Antibody test cassettes. Our feasibility study demonstrated that the developed device can sensitively detect and quantify the anti-SARS-CoV-2 Spike RBD IgG antibody within the clinically relevant concentration range. The reader can quantify IgG levels as low as 0.1μg/ml. While the detection and quantification performance of the developed system is similar to those of lab-based ELISA systems, the cost, size, and speed of the developed device (∼US$350; $8 \times 11 \times 10\text{cm}^3$; 15 minutes) are orders of magnitude superior to lab-based ELISA. The detection limit reported in this work is for spike RBD IgG antibody only, and as such we anticipate the limit of detection to be better for IgG developed from other types of proteins such as nucleocapsid. It should be noted that the developed portable and low-cost thermo-photonic device is the outcome of our gradual efforts in the past few years in lowering the size and cost of active thermography systems and thermo-photonic interpretation of LFAs. Technical information on these research and developments can be found in references [25]–[28]. While we intend to commercialize the developed low-cost technology in near future, individuals interested in the developed low-cost and portable technology are urged to contact the corresponding author for the possibility of obtaining the device through academic collaboration.

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