Computer Vision-aided CRISPR Diagnostics for the Detection of COVID-19

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***Abstract*—Surveillance testing is a key strategy to control the spread of COVID-19. Unlike the gold standard testing method, quantitative reverse transcription polymerase chain reaction (RT-qPCR), CRISPR diagnostics have recently become a more appealing alternative as they are proven to be faster, simpler, and more affordable. However, the current CRISPR diagnostic readouts are typically non-quantitative, making them error-prone and lacking crucial information of viral load. To further improve the CRISPR diagnostic method, we have developed a custom computer vision algorithm that works in complement to common transilluminators to process fluorescence images of the diagnostic samples, quantify their fluorescence signals, and assign the test results. Our analysis showed that the quantified fluorescence intensity was directly correlated to the sample viral load, useful information for transmissibility and disease severity. Verified through laboratory and clinical samples, our algorithm accurately discriminated the samples with the viral RNA as low as 6.25 copies/uL, and correctly classified nasopharyngeal swab (NP swab) samples with 100% accuracy. Our work serves as a potential technique to improve the accuracy of CRISPR diagnostics of COVID-19 and promote rapid testing vital to the containment of the ongoing pandemic.**

Keywords—COVID-19, SARS-CoV-2, CRISPR, Diagnostics

I. Introduction

COVID-19 is caused by an infection of the novel coronavirus known as SARS-CoV-2. Over the past year, the virus has infected more than 100 million people and led to over 2 million deaths. The death tolls have continued to rise with no end in sight. One strategy proven to be effective in containing the spread of the virus is to conduct surveillance testing to catch infected individuals early on and quarantine them before transmitting the virus to others. However, the current gold standard method for testing, known as quantitative reverse transcription polymerase chain reaction (RT-qPCR), is relatively costly, slow, and laborious. It also requires advanced equipment available only at major laboratories or hospitals. Such limitations restrict testing to major city areas, limit testing capacity, and become main hurdles for surveillance testing in which large, rapid, and on-site testing is required.

CRISPR diagnostics have emerged as a novel molecular diagnostic technique that is low-cost, fast, and simple [1]–[4], while maintaining its sensitivity and specificity to be on par with those of RT-qPCR. Nonetheless, CRISPR diagnostics are currently restricted by their non-quantitative readouts [5]–[7]. This is disadvantageous because quantitative readings can offer crucial information such as viral load that is shown to be an indicator of disease severity and infectiousness [8], [9]. To enable the quantitative readouts, we have developed a computer vision-based method that analyzes fluorescence images of the CRISPR assays, determines the test results, and quantifies the test signals. Therefore, this study addresses the main limitations of the CRISPR diagnostic platform and enables its applications to large COVID-19 screenings outside of laboratory settings.

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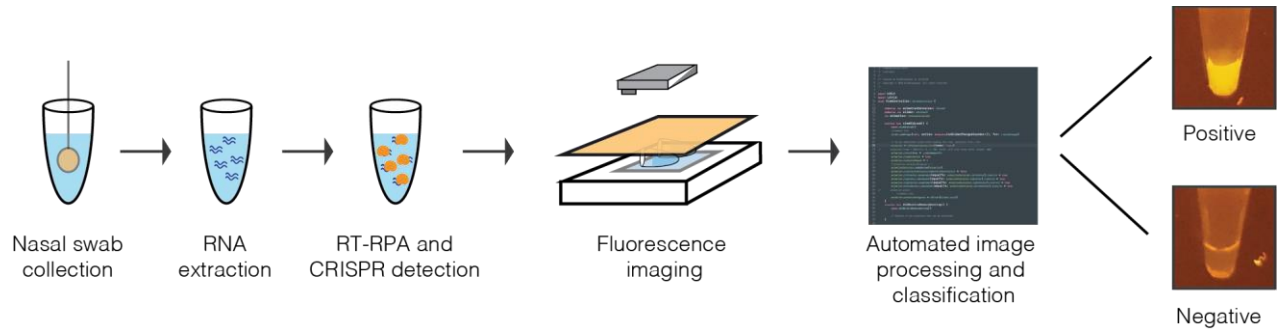

Fig. 1. The overall process of the COVID-19 detection using the CRISPR diagnostic platform together with our custom computer vision algorithm.

To perform the test, viral RNA is extracted from nasopharyngeal swabs and amplified using a method called reverse transcription recombinase polymerase amplification (RT-RPA). The amplified samples are then transferred to the CRISPR detection reagent for identifying the presence of SARS-CoV-2 genetic material. CRISPR reaction mix consists of a guide RNA (gRNA), Cas12a enzyme, and fluorophore-quencher probes. The gRNA determines the specificity of the test by recognizing and hybridizing to the target DNA sequence in the samples. Once a target is found, Cas12a enzyme becomes activated and in turn indiscriminately digests any DNA sequence, including the fluorophore-quencher probes. As a result, the fluorophore is released from the quencher and free to emit fluorescence [10], [11]. After the CRISPR reaction is completed, samples are placed under a low-cost commercially available transilluminator for fluorescence imaging. Fluorescence images can be acquired with a smartphone or a camera and processed through our custom software. Our algorithm will determine if a given sample is positive or negative and report a quantitative value representing a viral load within the sample (Fig. 1). The entire test costs $10 per sample and can be completed within 45 minutes. This is 4-5 times faster and more affordable than the gold standard, RT-qPCR [10].

II. Materials and Methods

A. Image processing and analyses

Laboratory or clinical samples contained in 0.1mL PCR strips were processed and diagnosed through the CRISPR detection procedure detailed in II.C-II.D. Once the reactions were completed, the strips were placed under a blue light transilluminator (BIO-HELIX, BP001CU) for fluorescence imaging. A smartphone with a fixed camera setting was used to acquire fluorescence images of the samples and send the images over for image analysis. The phone was laid on top of a custom imaging stand to keep the imaging distance consistent and provide stability for best quality images.

To process the acquired fluorescence images, a Python script was written to first identify the region corresponding to the liquid component of each tube. The image was edge detected and segmented using Felzenszwalb's graph-based segmentation. Further filtering on the segment area, intensity and location was implemented to select for the eight segments representing the true fluorescence regions of the strip to be analyzed. The centroid of each region was identified, and a square of 50x50 pixels was defined around each region and designated as a region of interest (ROI) for the measurement of fluorescence signals. Two methods of signal analyses in the two color systems, including Red, Green, Blue (RGB) and Hue, Saturation, Value (HSV), were examined (Fig. 2).

Because the probes utilized in the CRISPR reaction emit fluorescence that spans the wavelength of 500 – 650nm, we calculated the RGB-based fluorescence by averaging the red and the green channels of the identified ROIs. For the HSV-based system, the images were projected onto the HSV color space, and the hue averages of the ROIs were calculated as a measure of fluorescence signal. The signals derived from the two systems were compared in II.B and II.C.

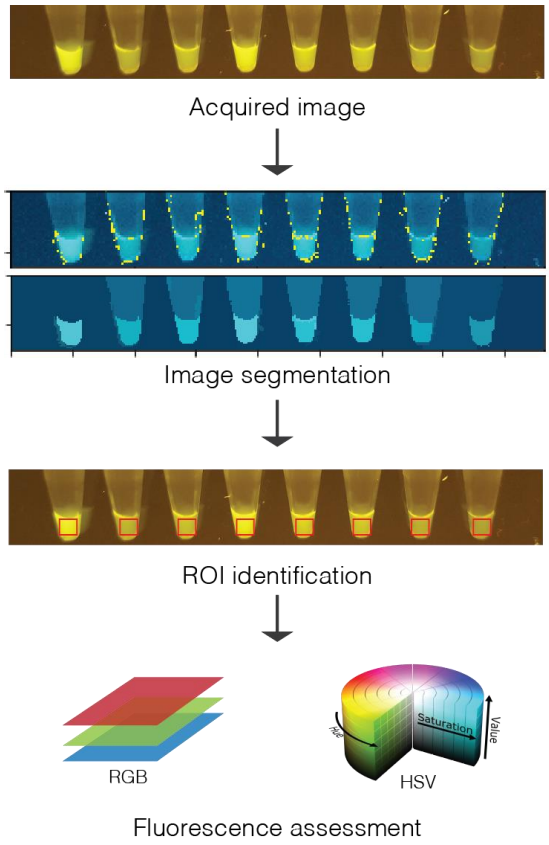

Fig. 2. Overall workflow of the image analysis algorithm.

B. Correlation analysis of the fluorophore concentration and the fluorescenc signal

The fluorophore, 6-Carboxyfluorescein (6-FAM), with no quencher was serially diluted into concentrations between

0.02 – 1.5 uM. Images of solutions with these fluorophore concentrations were acquired with the transilluminator and analyzed with our custom scripts using the RGB and HSV color models. The signals obtained from the two models at different dye concentrations were plotted in fig. 3.

C. CRISPR detection of laboratory samples

Synthetic RNA templates encoding a portion of SARS-CoV-2 S gene were *in vitro* transcribed from a plasmid using T7 Riboprobe *in vitro* transcription systems (Promega, P1440). The templates were serially diluted into five varying concentrations - 1000, 125, 62.5, 12.5, 6.25 copies/uL– which then served as starting samples for the tests. Each sample was respectively reversed transcribed and amplified using RevertAid Reverse Transcriptase (Thermo Scientific, EP0442) and TwistAmp Basis Kit (TwistAMP, TABAS03KIT) according to the manufacturers' protocols. The amplified samples were subsequently detected using the CRISPR-Cas12a detection reagent (NEB, M0653T). The fluorescence images were acquired for each template concentration at 10, 20, and 30 minutes after the initiation of the CRISPR-Cas12a reaction. Three replicates were carried out for each template concentration.

D. CRISPR detection of clinical samples

Ten nasopharyngeal swabs were collected at Rajavithi hospital, Bangkok, Thailand in 2020. The samples were subjected to RNA extraction using GenUP Total RNA Kit (BiotechRabbit, BR0700902). 1uL of the extracted samples were added to the detection reactions in place of synthetic templates described in II.C.

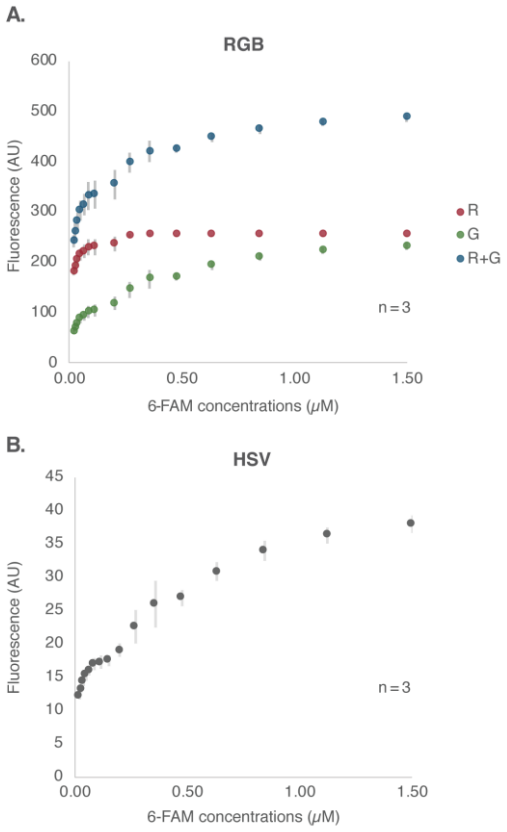

Fig. 3. Fluorescence readouts through RGB and HSV color systems at a relevant range of fluorophore concentrations.

III. Results and Discussion

A. Correlation analysis of the fluorophore concentration and the fluorescenc signal

We first investigated the signals obtained from the RGB color model. We found that the signals from the red color channel were higher than those from the green channel. However, the intensity in the red channel appeared saturated at high dye concentrations. Further assessment showed that combining the intensity from the red and the green channel led to better signal sensitivity across the entire range of fluorophore concentrations (Fig. 3A). The signals obtained from the hue average showed a similar trend of sensitivity and overall coefficient of variations (Fig. 3B).

B. CRISPR detection of laboratory samples

Next, we evaluated the two algorithms on the fluorescence images acquired from the CRISPR detection of synthetic RNA templates. Five concentrations of RNA templates encoding S gene of SARS-CoV-2 and a negative control with no template were subjected to the CRISPR detection process

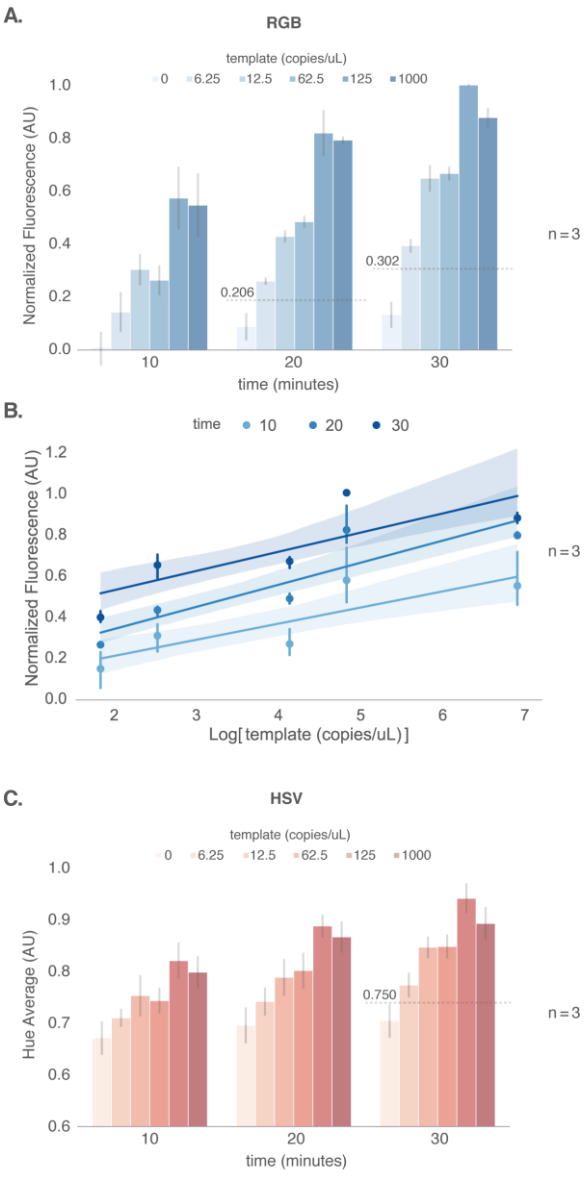

Fig. 4. CRISPR detection of SARS-CoV-2 using synthetic RNA templates.

detailed in Figure 1. The fluorescence images were acquired at 10, 20, and 30 minutes into the CRISPR reaction and sent for analyses through the RGB-based and the HSV-based algorithms. With RGB-based analysis, we observed increases in fluorescence signals with the template concentrations. A small signal difference between the lowest template concentration and the negative control was observed at 10 minutes, and the distinction became statistically significant at 20 minutes. The confidence level improved to more than 99% at 30 minutes (Fig. 4A). This analysis suggests that our test has the limit of detection (LoD) of 6.25 RNA copies/uL. In addition, our correlation analysis revealed a log linear correlation between the template concentration and the fluorescence value (Fig. 4B), suggesting that our fluorescence value can serve as a direct indicator of the viral amount in the tested sample.

Our HSV-based algorithm yielded a similar trend of signals. However, we could discriminate the positive signals from the negatives at 30 minutes, 10 minutes slower than the RGB-based analysis (Fig. 4C). Therefore, we proceeded with the RGB model for our subsequent experiments.

A prominent distinction of our reaction lay within our gRNA that selectively targets a portion of the SARS-CoV-2 S gene [10], altered from the prevalent N gene which is the target region recommended by the World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) [12], [13]. Unlike the N gene that tends to give an all-or-none response [5], our detection targeting S gene exhibits gradient signals that correlate to the concentration of the starting RNA template. The correlation permits quantification of the viral amount in the tested sample which was less feasible with the traditional N gene target.

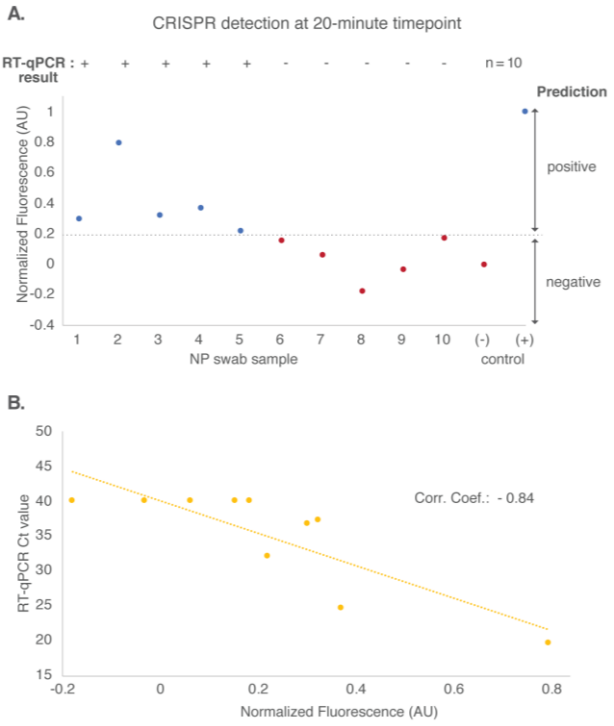

Fig. 5. CRISPR detection of SARS-CoV-2 in nasopharyngeal swab samples.

C. *CRISPR detection of laboratory samples*

We further evaluated our test on ten nasopharyngeal swab (NP swab) samples collected at Rajavithi hospital, Thailand. The samples were subjected to RNA extraction followed by a similar test procedure as the synthetic templates. After 20 minutes of CRISPR detection reaction, the samples were fluorescence imaged and analyzed with our RGB-based algorithm. Using the criterion found with the laboratory templates, our algorithm correctly classified 100% of the clinical samples (Fig. 5A) including the samples with the RT-qPCR Ct value as high as 37.18. Furthermore, our fluorescence score strongly correlated with the RT-qPCR Ct value with the correlation coefficient of 0.84 (Fig. 5B). This strong relationship indicates that our fluorescence can be translated to the prevalent Ct value.

IV. Conclusions

We have developed a computer vision algorithm that processes and classifies fluorescence images from the CRISPR diagnostic of COVID-19. The algorithm has been verified to accurately classify 100% of both laboratory and clinical samples. Furthermore, our system offers a quantitative fluorescence score that correlates to the amount of viral RNA and the RT-qPCR Ct value. Such information of viral load is proven to be an important indicator of disease severity and contagiousness, information that traditional CRISPR diagnostics are unable to obtain. Hence, our method addresses the main shortcomings of the CRISPR diagnostic platform and promotes its use for rapid, affordable, and on-site testing of COVID-19 that is critically in need in the ongoing pandemic.

Acknowledgment

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Ethical Statement

This study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (IRB No. 302/63).

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