Electronic Supplementary Material (ESI) for Analyst.


# Advancing Diagnostic Efficacy using a Computer Vision-Assisted Lateral Flow Assay for Influenza and SARS-CoV-2 Detection

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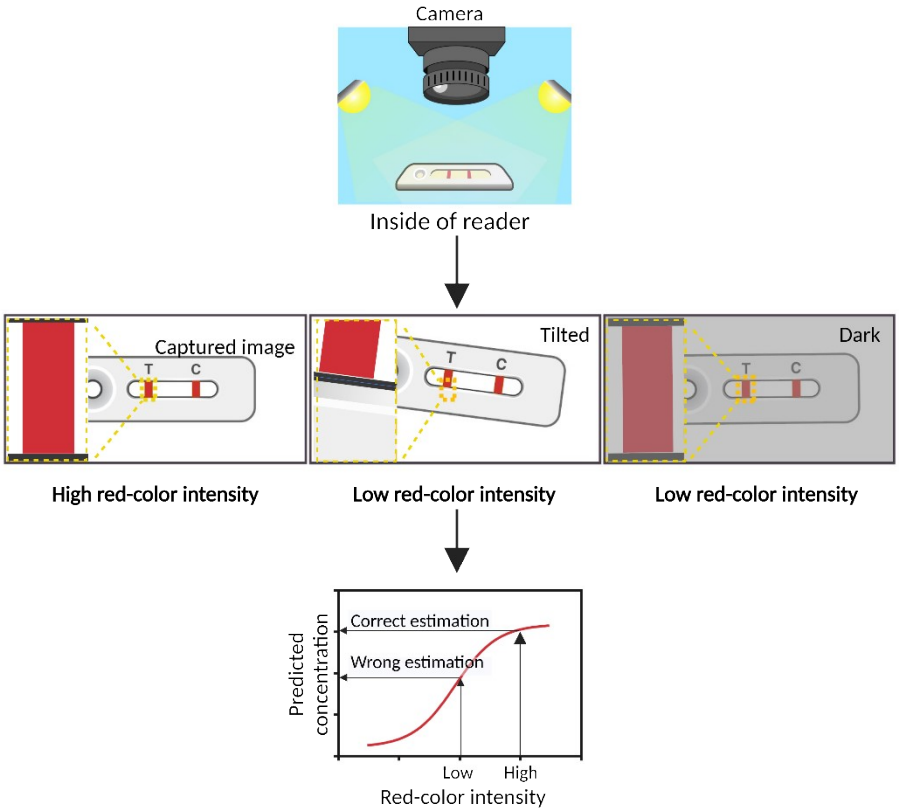


**Fig. S1.** Erroneous estimation can occur during the process of image acquisition. Incorrect positioning of the LFA kit (tilt or shift) or external conditions (such as lighting) can influence the intensity extraction, leading to inaccurate predictions.

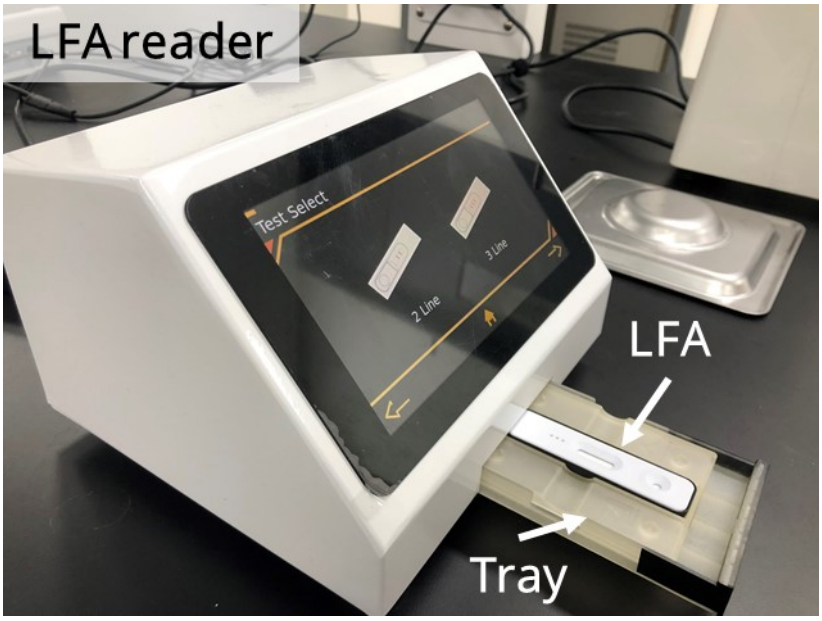


**Fig. S2.** Image of an LFA reader.

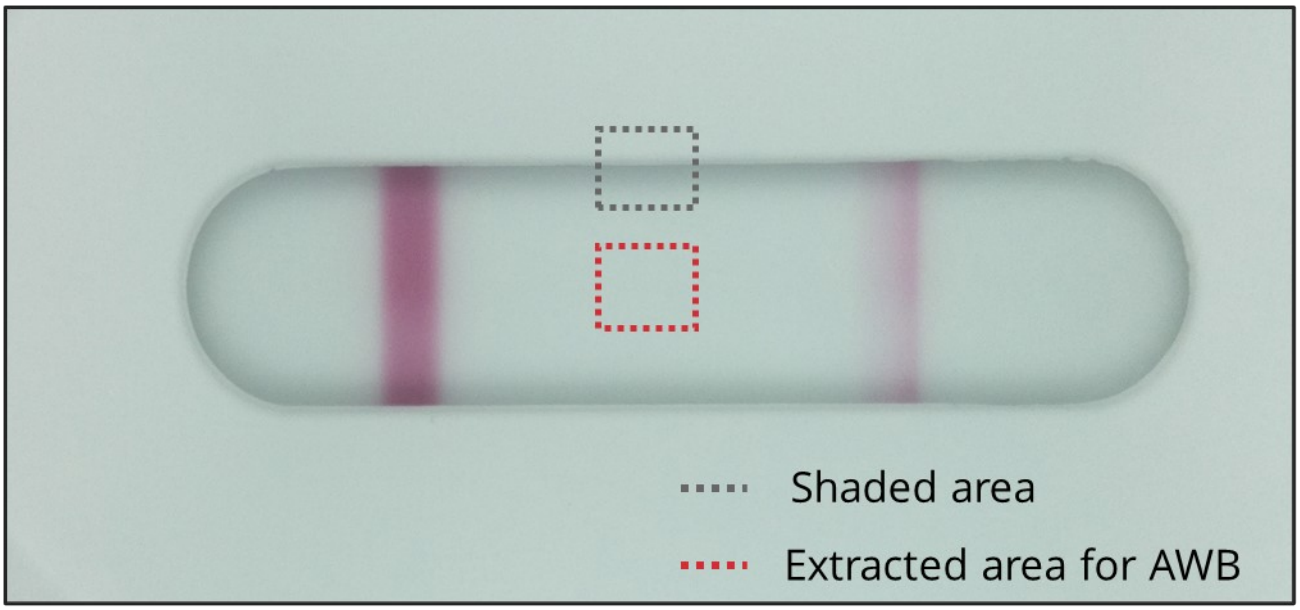


**Fig. S3.** Extracted region for white balancing. As the membrane is located below the cassette window, when it is closer to the boundary of the window, the possibility of it being affected by shadows is high. Therefore, the middle domain of the membrane was used.

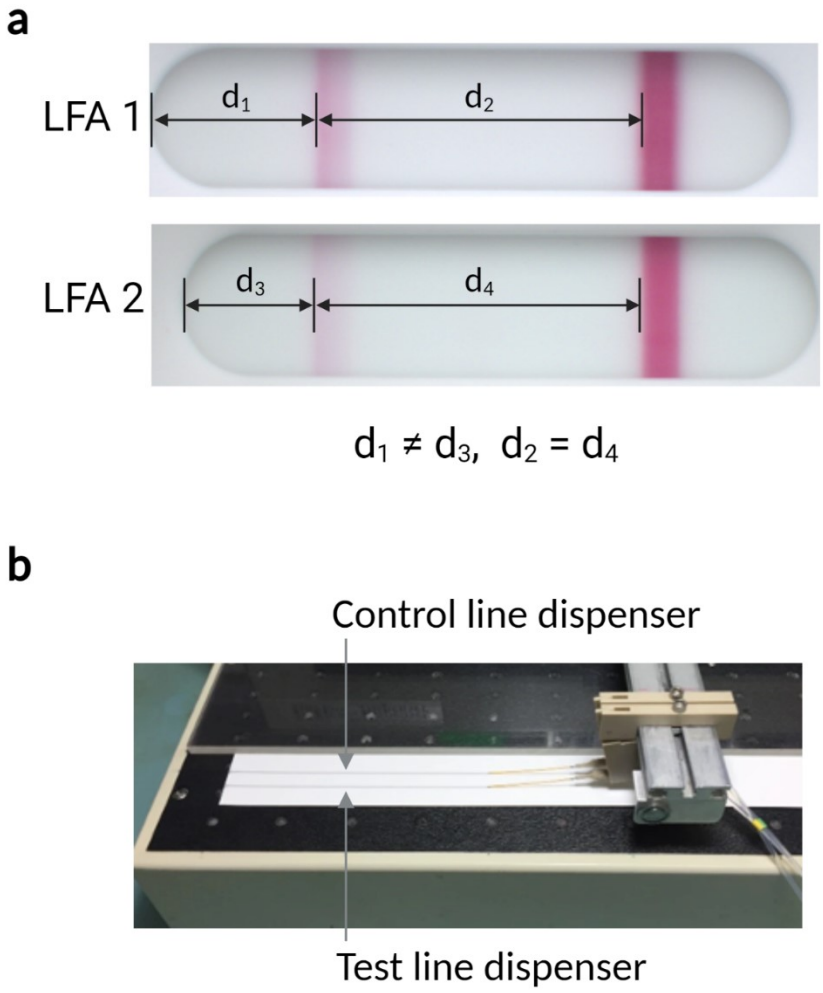


**Fig. S4.** a) Two images show the mismatch of the test line and control line locations when measured from the left end of the cassette contour in a commercial LFA. The distance from the left cassette contour to the test line showed different values when the cutting process was misaligned. Meanwhile, the distance was kept constant between the test and control lines regardless of the LFA kit. b) Since the distance between the test and control lines was kept constant with the use of a dispenser, we calculated the position of the test line using the constant distance from the control line.

**Table S1.** Clinical sample information

| Number | Sample type | Age | Gender | Ct value | Result |
|---|---|---|---|---|---|
| #1 | NP/OP | 44 | Male | 18.7 | Positive |
| #2 | NP/OP | 33 | Female | 22.8 | Positive |
| #3 | NP/OP | 46 | Male | 26.8 | Positive |
| #4 | NP/OP | 78 | Female | 28.1 | Positive |
| #5 | NP/OP | 26 | Male | 38.3 | Negative |

**Test line intensity acquisition**

When we demonstrated the intensity of the test line, we used the red and blue intensity ratio because the intensity of a single color largely depends on the brightness of the light. To reduce the effect of the brightness of illumination, the ratio of red to blue colors was used. The reason why we choose red and blue is that as the test line reacts, red intensity is constant or marginally decreased[1], and the blue intensity is more tolerant to external light sources than the green intensity[2].

An image is generally expressed in three color channels (R, G, B), and each pixel is expressed in 8 bits; consequently, the pixel numbers range from 0 to 255 ($2^8 = 256$). Ideal white has [R, G, B] = [255,255,255] value. As the membrane transitions to red, the red channel values are relatively sustained, whereas the blue and green channel values diminish. Assuming the membrane approaches an ideal white state through the white balance algorithm's assistance, the calculation of the difference is achieved by subtracting the acquired value from 255.

Consequently, we demonstrate the intensity of the test line as shown below.

$$x = \frac{\Delta R}{\Delta B} = \frac{255 - R}{255 - B} \qquad (2)$$

Moreover, when we plotted the graphs shown in Figs. 5 and 6, we subtracted the intensity $x$ by the intensity of concentration 0 ng/ml.

$$\Delta \text{ intensity} = x - x_{conc.\ 0\ ng/ml} \qquad (3)$$

**Reference**


1. J. Park, *Sensors*, 2018, **18**, 4084.
2. M. Sonka, V. Hlavac and R. Boyle, *Image processing, analysis, and machine vision*, Cengage Learning, 2014.