Electronic Supplementary Material (ESI) for Analyst.

Image Analysis for Microfluidic Paper-Based Analytical Device Using the CIE *L*a*b** Color System

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Materials and Reagents

A universal pH indicator was prepared by mixing 0.11 mM thymol blue (pKa_1 = 1.7, pKa_2 = 8.9, pH range 1.2 - 9.6, Wako Pure Chemical Industries, Ltd., Osaka, Japan), 0.45 mM methyl red (p*K*a = 5.1, pH 4.2 - 6.2, Wako Pure Chemical Industries, Ltd.,), 0.96 mM bromothymol blue (p*K*a = 7.1, pH 6.0 - 7.6, Kanto Chemical Co., Inc., Tokyo, Japan), and 3.1 mM phenolphthalein (p*K*a = 9.3, pH 7.8 - 10.0, Kanto Chemical Co., Inc.) in 95% ethanol.[1] 0.1 M phosphate buffer (Wako Pure Chemical Industries, Ltd.), 0.1 M acetic acid buffer (Kanto Chemical Co., Inc.), and 0.1M Tris-HCl buffer (Wako Pure Chemical Industries, Ltd.) were used for adjusting pH value in test solutions in a range of 2 to 9.

Fabrication of Paper Devices

A screen-printing table (WHT No. 3 and equipped with a vacuum pump that was manufactured by Mino Group Co., Ltd., Tokyo, Japan), a screen stencil (T-200 nylon mesh on an aluminum frame), and a squeegee were purchased from Unno Giken Co., Ltd. (Tokyo, Japan). The screen stencil was designed with 36 paper device patterns in it. The detailed fabrication procedures were described in the literature.[2] Polydimethylsiloxane (PDMS, SILPOT 184 Kit, Dow Corning Toray Co., Ltd., Tokyo, Japan) was used as a polymer ink for hydrophobic patterning. Paper substrate selected for the device was Whatman chromatography paper #1 purchased from GE Healthcare (200 × 200 mm, thickness: 0.18 mm, GE Healthcare Japan Co., Ltd., Tokyo).

Image analysis

We used the ImageJ (ver. 1,48) software to obtain digital color information from digital images. A circular region of interest (ROI, 27000 pixels) was drawn around the detection zone in the digital image and was analyzed using the software, as shown in Fig. S1 (red

broken line). For RGB values and grayscale, the “Measure RGB” plugin was used. The paper-based device had 8 detection zones with 4-mm diameter. We analyzed the 8 detection zones for a single paper-based device. We performed the experiments using separate devices in triplicate. Fig. S2 shows a plot of grayscale at various pH values.

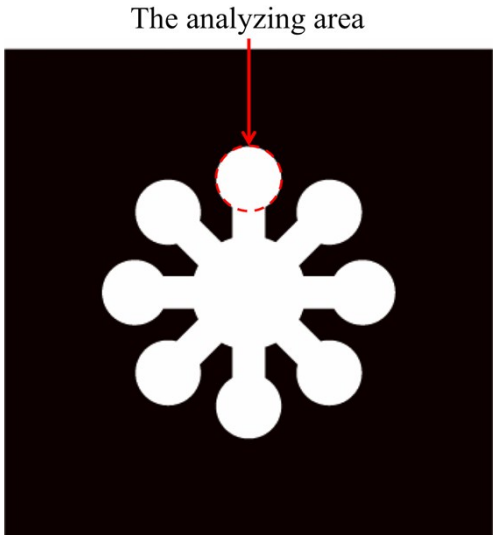

Fig. S1 The analyzing area of paper-based device.

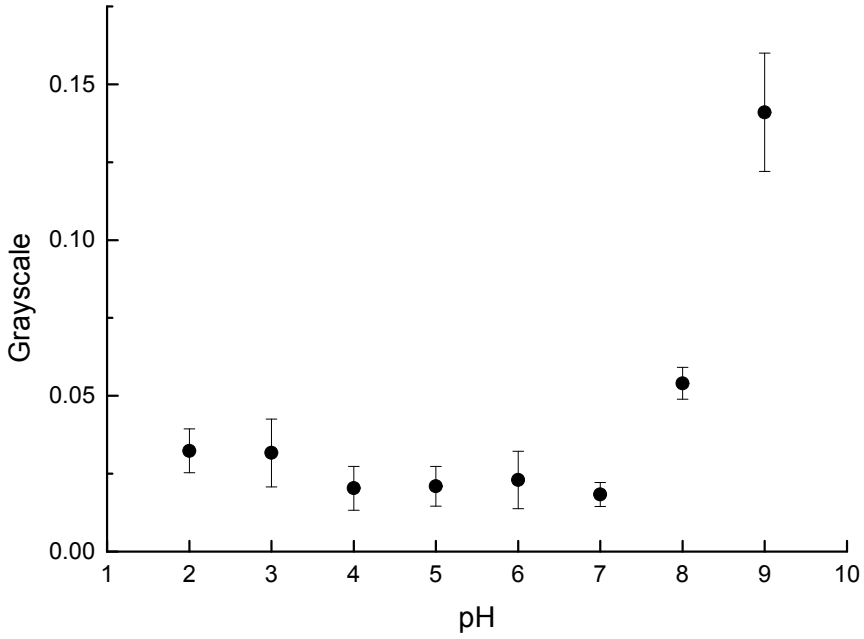

Fig. S2 Plot of grayscale against pH value.

Measurement of CIE *L*a*b color system coordinates**

The images were converted from the RGB color space to the CIE *L*a*b** color space and split to *L**, *a**, and *b** channel with the Color Space Converter plugin prior to the image analysis. The detection zones in the split images were selected with circle and measured to obtaine *L**, *a**, *b** values. Fig. S3 shows 3D and 2D plots in CIE *L*a*b** color space. Fig. S3 (b) shows a plot of *a** and *b** coordinates. Figs. S3 (a) and (b) indicate that the data points formed a trace in an arc in response to an increase in pH value. Fig. S4 shows plots of *L**, *a**, and *b** coordinates against pH value, and exhibited non-monotonic changes. Fig. S5 shows a plot of $\tan^{-1}(b^*/a^*)$ against the pH value.

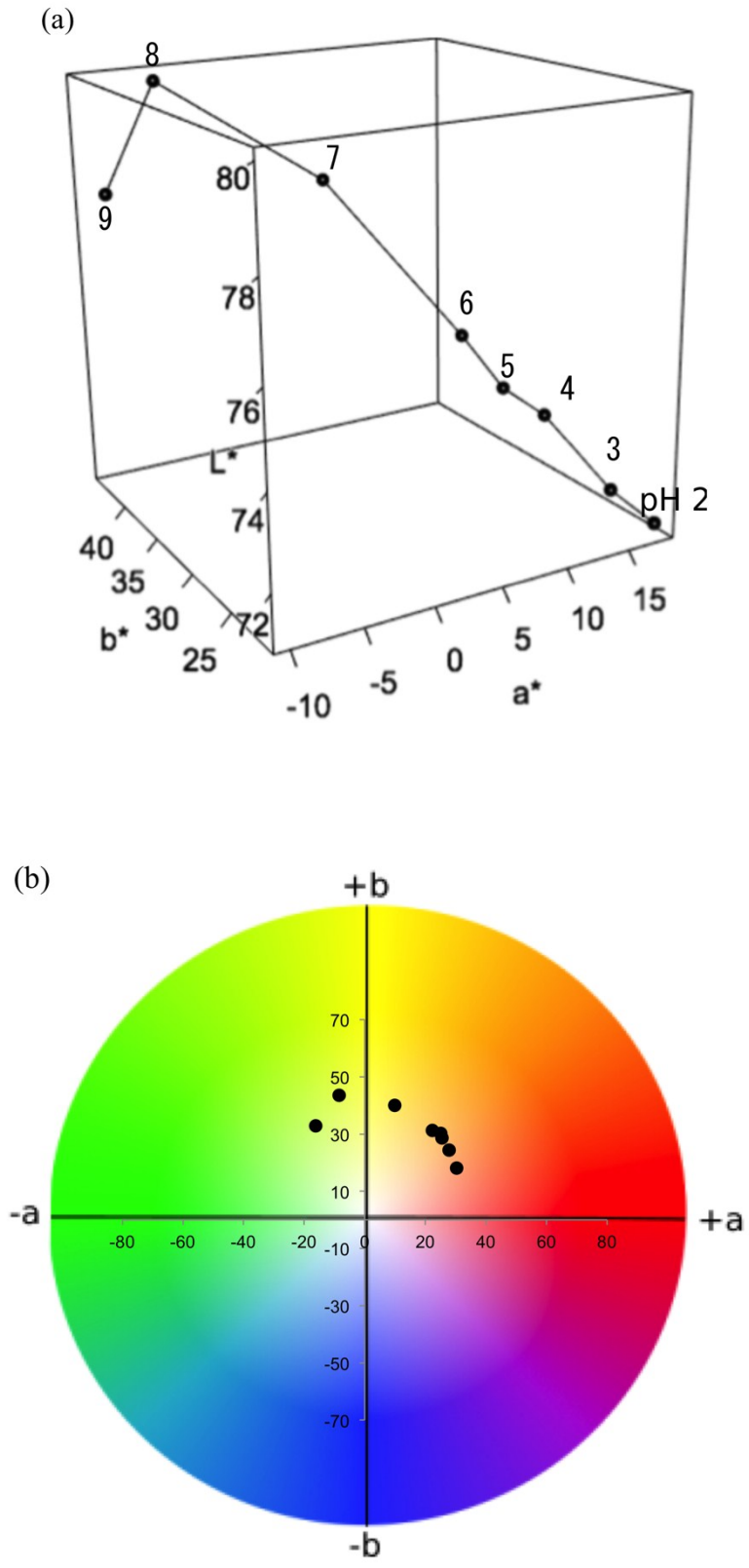

Fig. S3 (a) 3D and (b) 2D plot of various pH values in CIE *L*a*b** color system.

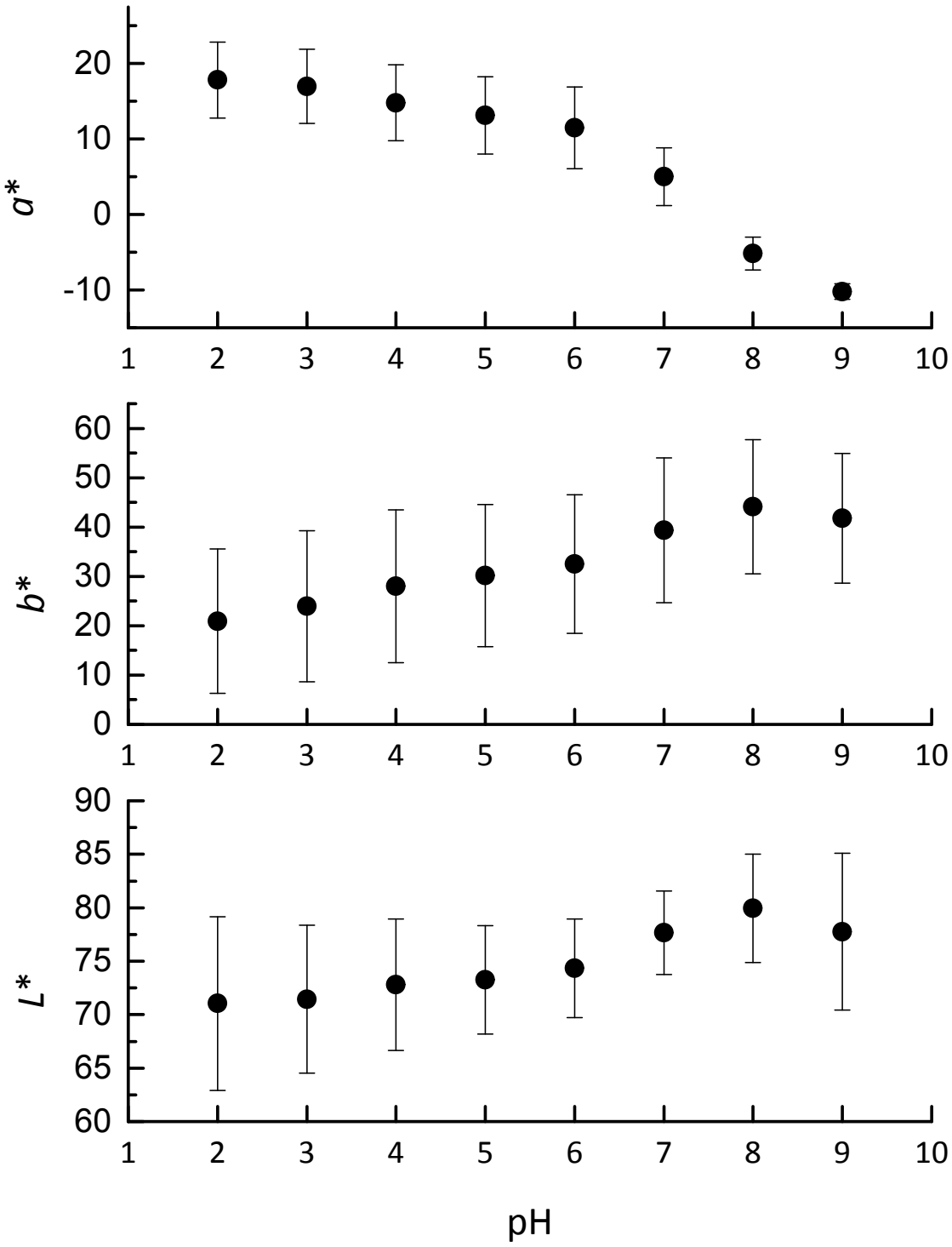

Fig. S4 Plots of $L^*a^*b^*$ against pH value.

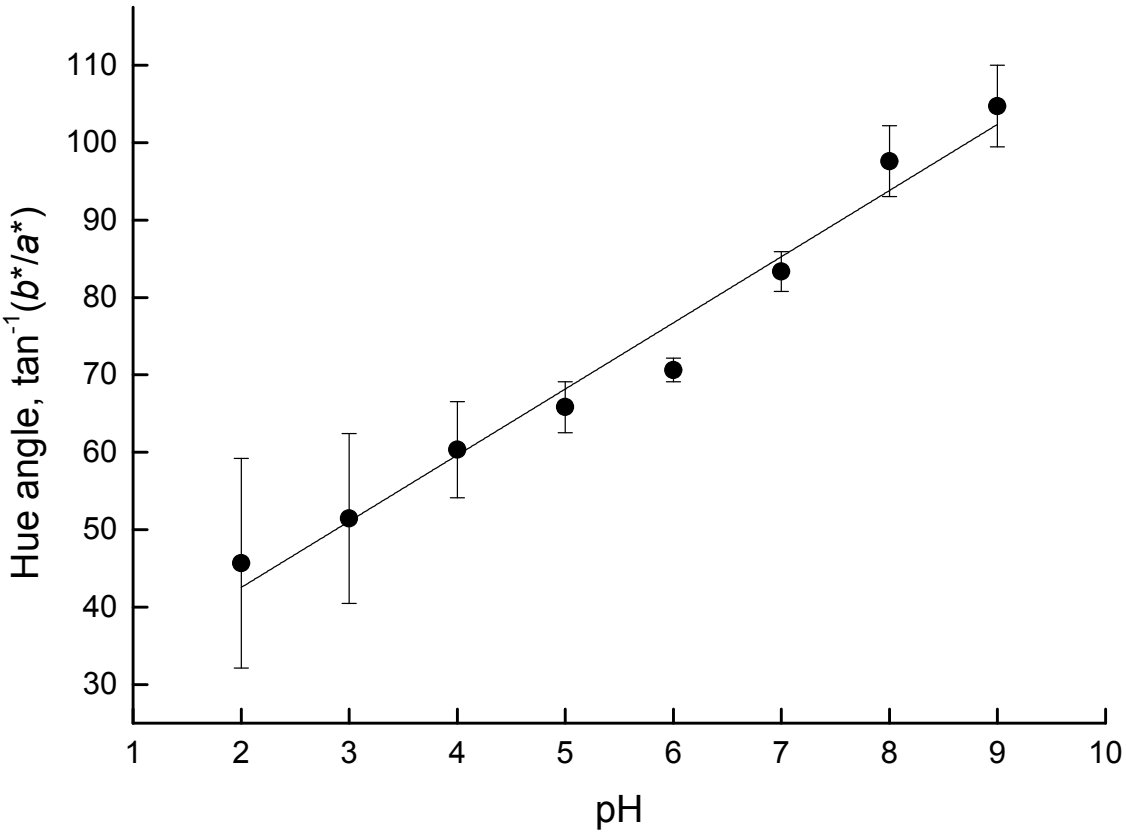

Fig. S5 Plot of $\tan^{-1}(b^*/a^*)$ at various pH values in CIE $L^*a^*b^*$ color system

References

1. K. Abe, K. Suzuki, D. Citterio, *Anal. Chem.*, 2008, **80**, 6928-6934.

2. S. Mohammadi, M. Maeki, R. M. Mohamadi, A. Ishida, H. Tani, and M. Tokeshi, *Analyst*, 2015, **140**, 6493-6499.