Supporting Information for

Traffic light type paper-based analytical device for intuitive and semi-quantitative naked-eye signal readout

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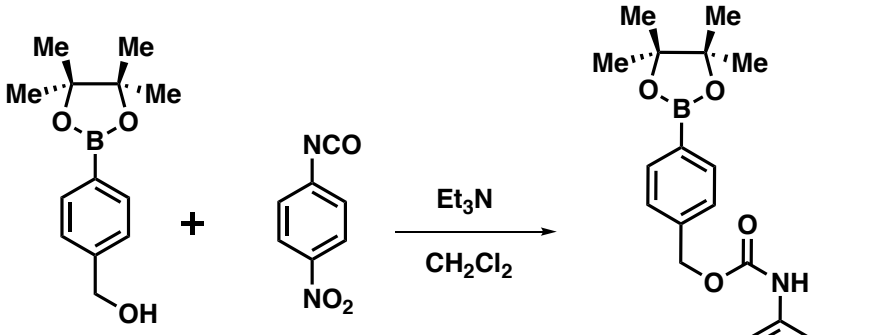
Scheme S1 : Synthesis of boronic acid pinacol ester derivative



4-(4, 4, 5, 5-Tetramethyl-1, 3, 2-dioxaborolan-2-yl) benzyl alcohol

4-nitrophenyl isocyanate

Boronic acid pinacol ester derivative

4-(4, 4, 5, 5-Tetramethyl-1, 3, 2-dioxaborolan-2-yl) benzyl alcohol (5.40 g, 23.0 mmol, 1.0 eq) was dissolved in triethylamine (3.3 mL, 23.8 mmol, 1.0 eq) under an Ar atmosphere at 0˚C. 4-Nitrophenyl isocyanate (4.20 g, 25.6 mmol, 1.1 eq) dissolved in dichloromethane (230 mL) was dripped into the solution over 20 min with stirring under Ar at 0˚C. Stirring was continued for 3 h at room temperature and the solvents removed by a rotary evaporator. The crude compound was purified by flash column chromatography (elution with Toluene-EtOAc 95:5) affording the boronic acid pinacol ester derivative as a white solid in 67% yield (6.15 g, 15.4 mmol).

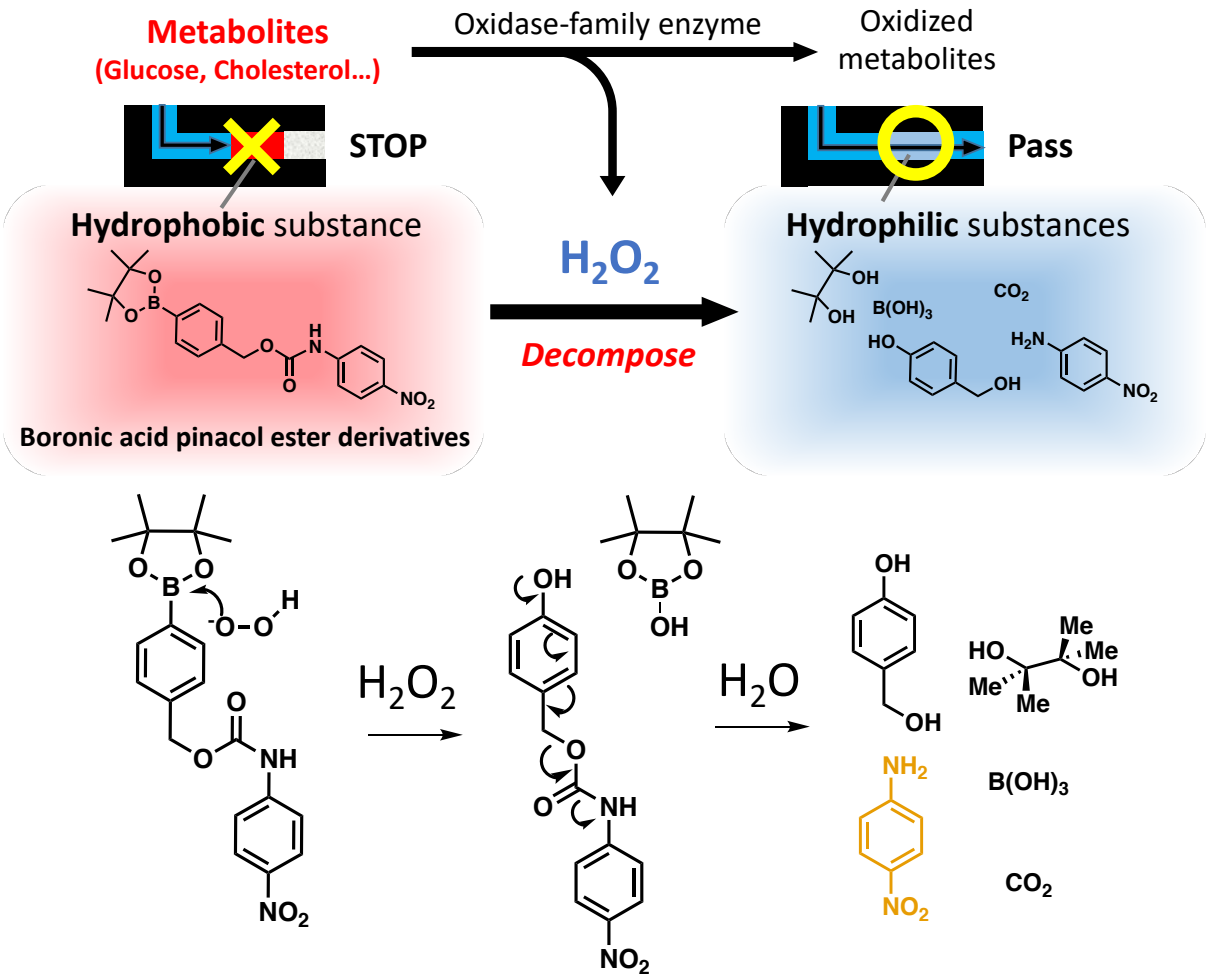
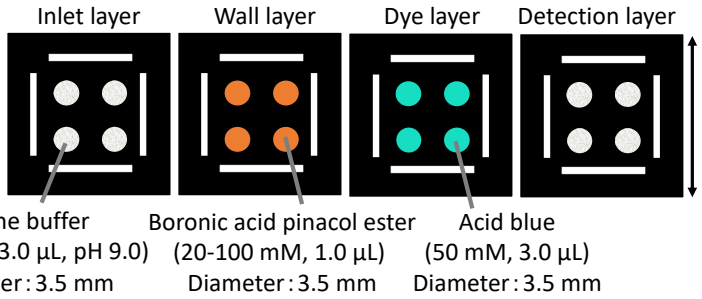


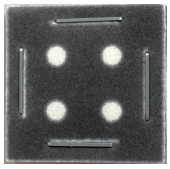
Fig. S1 : (a) Chemical valving system using a boronic acid pinacol ester derivative as phase-switching substance: the hydrophobic state stops the sample flow, while the hydrophilic state induced by decomposition following reaction with H_2O_2 allows the sample to flow through; (b) Mechanism of chemical decomposition of boronic acid pinacol ester derivative by reaction with H_2O_2, producing the yellow p-nitroaniline compound.

(a) Device design

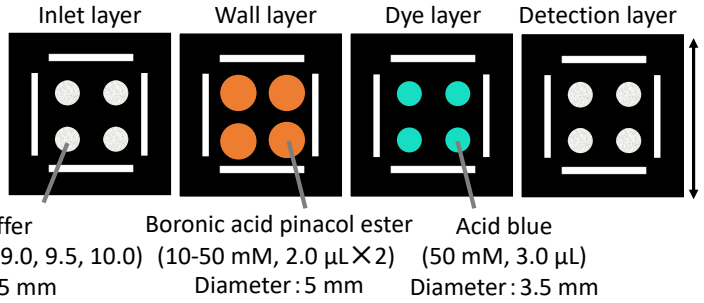
(1) Microcapillary (Device a)



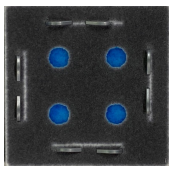
Top view



(2) Dispenser (Device b-e)



Back view



(b) Deposited reagents

Device name	Switching reagent application	Inlet layer(1)	Wall layer(2)	Dye layer(3)	Detection layer(4)	Applied sample
a	Microcapillary	Glycine buffer (500 mM, 3.0 µL, pH 9.0)	Boronic acid pinacol ester (20-100 mM, 1.0 µL)	Acid blue (50 mM, 3.0 µL)		H_2O_2 (0-100 mM, 10 µL)
b	Dispenser		Boronic acid pinacol ester (10-50 mM, 2.0 µL×2)			
c		Glycine buffer (500 mM, 3.0 µL, pH 9.0)	Boronic acid pinacol ester (50 mM, 2.0 µL×2)			H_2O_2 (0-50 mM, 10 µL)
d		Glycine buffer (500 mM, 3.0 µL, pH 9.5)				
e		Glycine buffer (500 mM, 3.0 µL, pH 10.0)				

(c) Device fabrication method

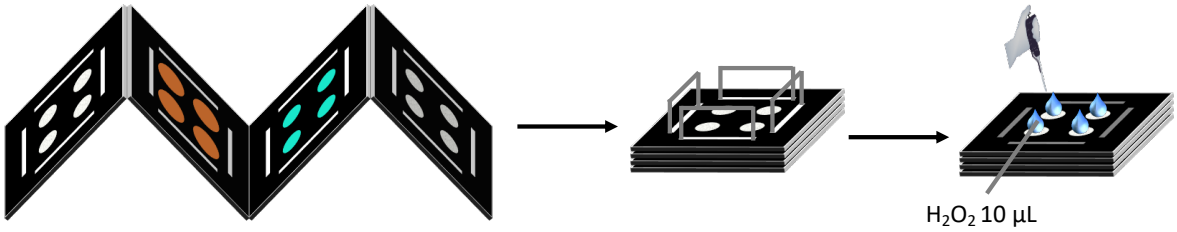


Fig. S2 : (a) Design of simple vertical flow origami-type paper device for flow-through time measurement. The indicated scales represent the pre-set values in the Adobe Illustrator CC software used for wax printing. Photograph of top and backside view of the device after color appearance. (b) Details about amounts and types of reagents applied to each layer and the application method of the boronic acid pinacol ester solution on the "wall layer" for Devices a-e. (c) Device fabrication and experimental method for flow-through time measurement.

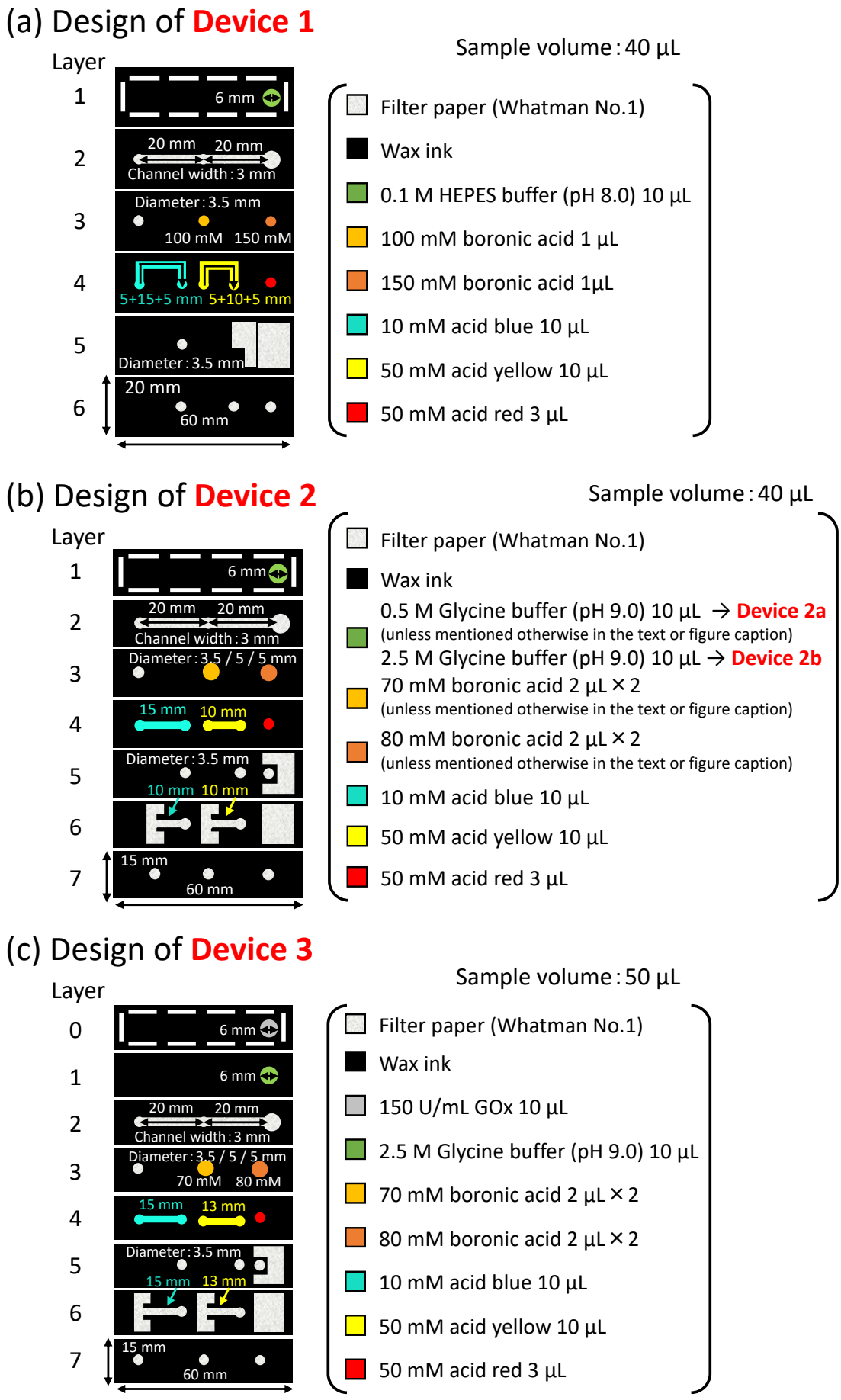


Fig. S3 : Design details of various traffic light type µPADs: (a) Device 1, (b) Devices 2a, 2b, (c) Device 3. The indicated scales represent the pre-set values in the Adobe Illustrator CC software.

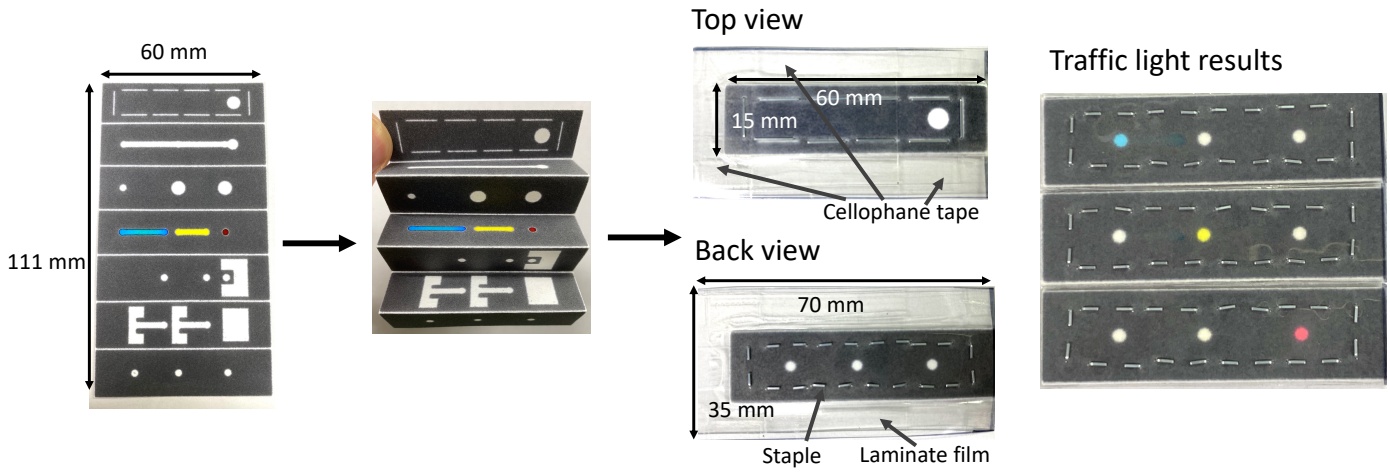


Fig. S4 : Traffic light type μPAD assembly method (shown on the example of Device 2) and photographs of device backsides showing the traffic light signal (green, yellow or red) after the application of samples of various H_2O_2 concentrations.

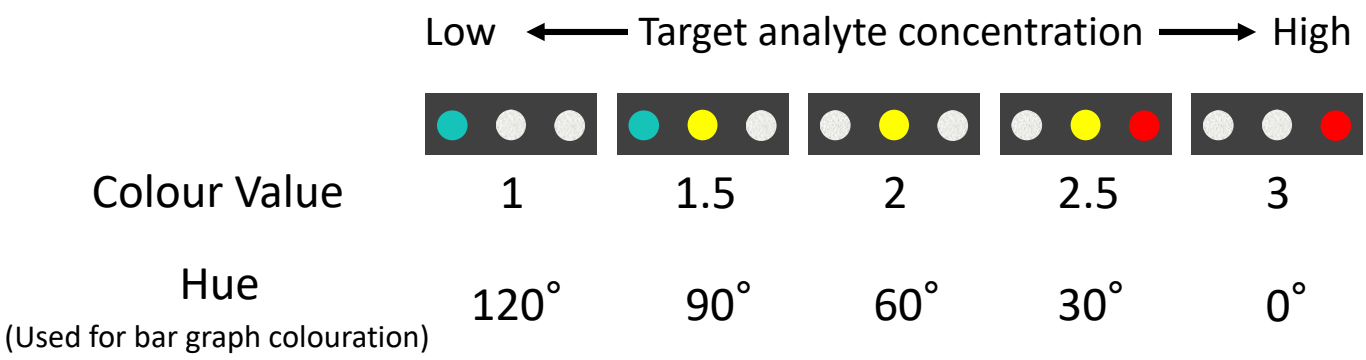


Fig. S5 : Schematic illustration of the colour evaluation method to convert the traffic light colours into color values. The hue is used to support the visualization of quantitative results at a certain analyte concentration.

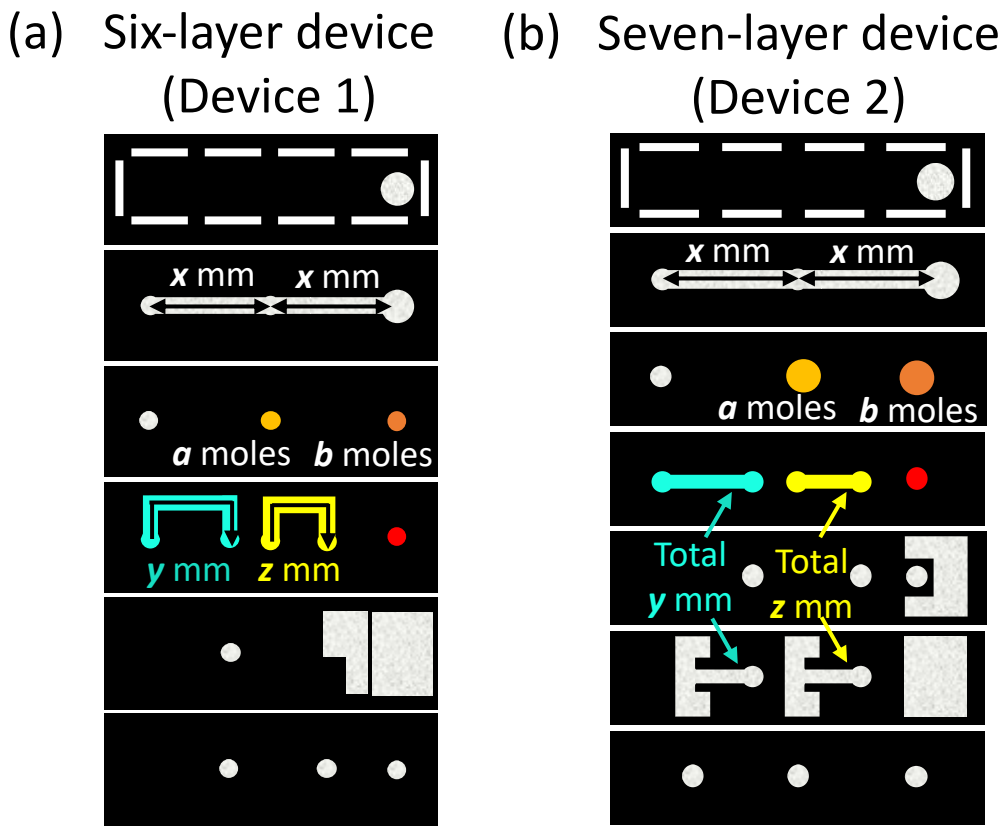


Fig. S6 : Devices of Fig. S3 with indication of variables used in equations (1) - (3) of the main text: (a) six-layer (Device 1) and (b) seven-layer (Device 2) traffic light type μPAD.

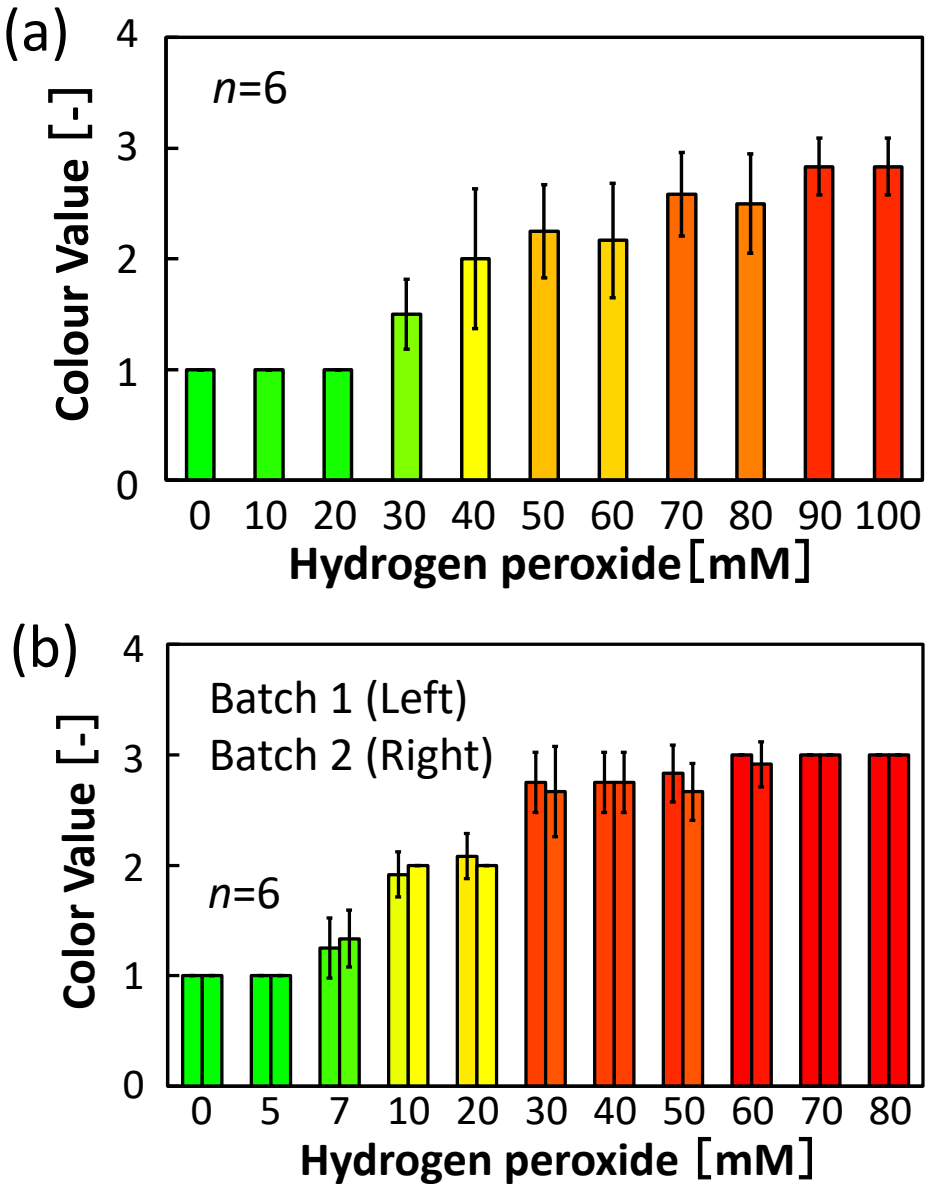


Fig. S7 : Results of traffic light assay for H_2O_2 detection using (a) Device 1, and (b) Device 2a demonstrating the reproducibility between two batches of devices independently fabricated within approximately 8 months. Error bars represent mean values ±1 σ for 6 individually fabricated μPADs.

(a) Microcapillary (Device a in Fig. S2)

Flow-through time [s]		Hydrogen peroxide [mM]					
		0	20	40	60	80	100
Boronic acid pinacol ester [mM] 1 μL	20	3±1	4±1	2±0	3±1	3±1	2±1
	40	14±10	7±4	38±60	6±3	6±2	6±1
	60	493±872	94±153	72±68	33±27	85±67	62±75
	80	978±952	233±215	259±150	168±133	83±76	131±76
	100	1402±797	581±98	249±107	300±37	192±91	206±88

(b) Dispenser (Device b in Fig. S2)

Flow-through time [s]		Hydrogen peroxide [mM]					
		0	20	40	60	80	100
Boronic acid pinacol ester [mM] 2 μL× 2 times	10	17±10	7±2	5±1	5±1	5±1	4±1
	20	287±132	23±9	18±3	20±7	15±1	13±2
	30	692±154	89±22	42±14	30±5	32±2	33±3
	40	1656±145	128±14	92±10	70±6	63±8	57±6
	50	>1800	202±11	113±14	103±4	86±18	81±14

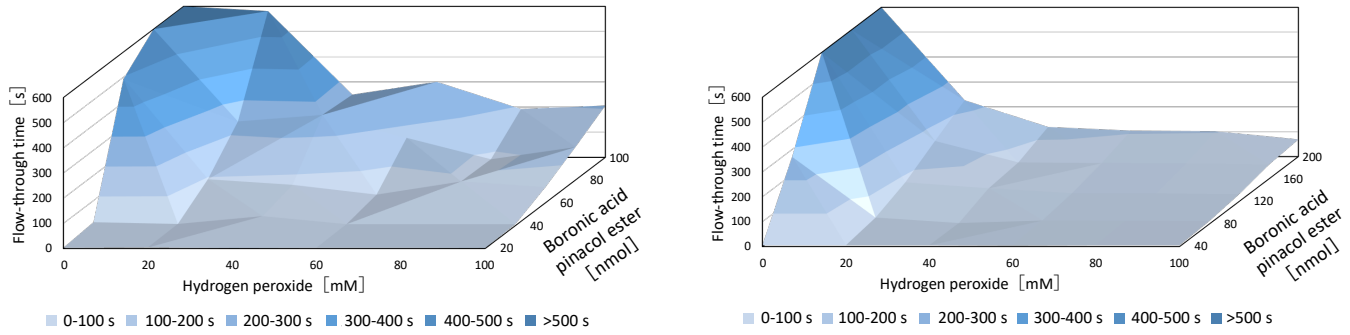
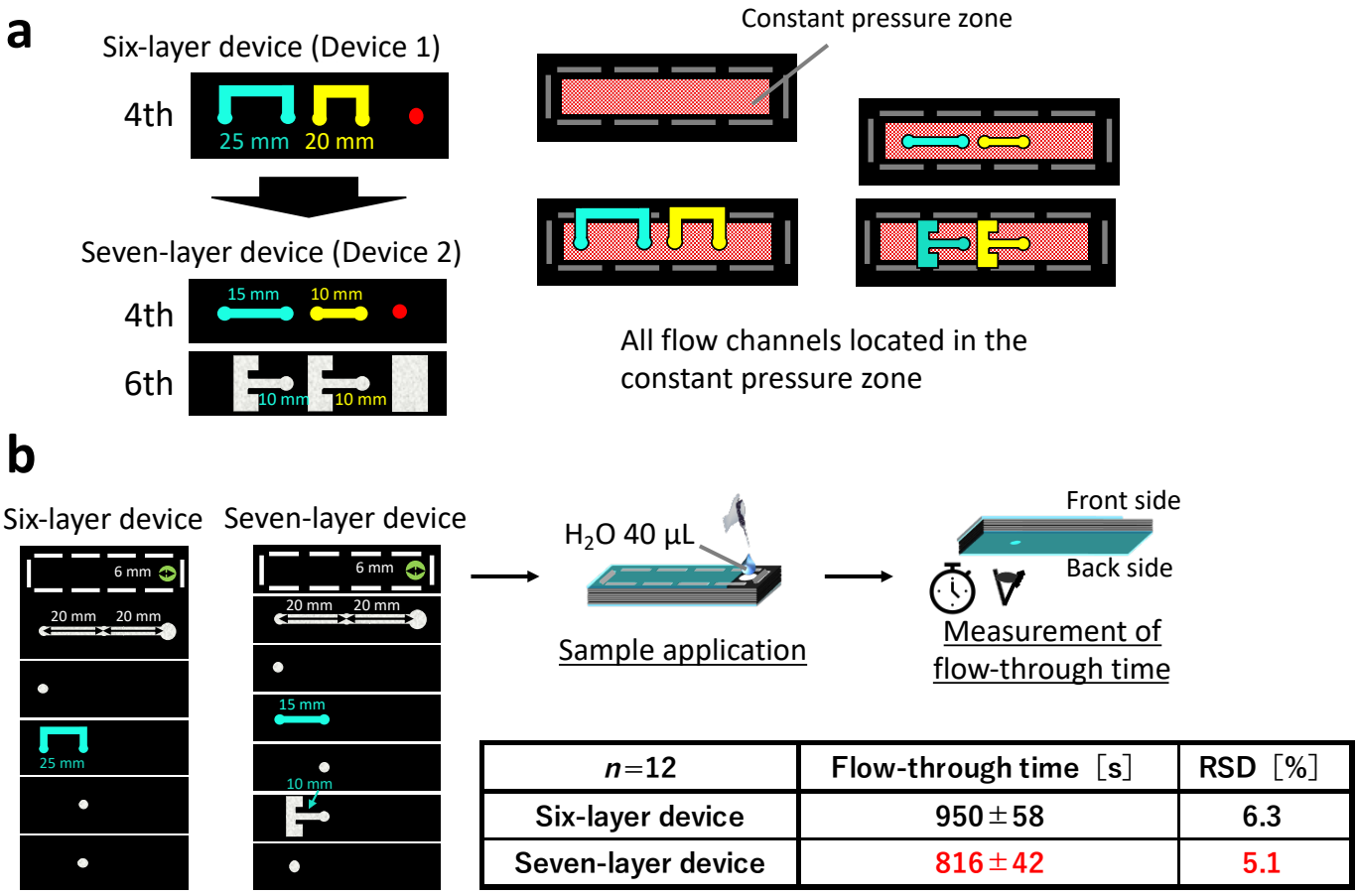


Fig. S8 : Measurement of H_2O_2 concentration-dependent flow-through times for model devices a and b (Fig. S2) to evaluate the fabrication reproducibility of hydrophobic walls. Fixed volumes of boronic acid pinacol ester derivative solutions of varying concentrations were deposited onto paper spots using (a) a microcapillary to apply the reagent solutions to the top surface of the wall layer (1 μL/spot), or (b) a dispenser to apply the reagent solutions to the top (2 μl/spot) and bottom surfaces (2 μl/spot) of the wall layer. The tables show the flow-through times from H_2O_2 sample application to colour appearance measured for Device a (microcapillary) and Device b (dispenser) ($n = 4$; mean value ± 1σ). Colours of table cells indicate the order of the flow-through time and are represented in a gradient using six levels of blue. The 3D-plots provide a more visual representation of the data shown in the tables.



n=12	Flow-through time [s]	RSD [%]
Six-layer device	950±58	6.3
Seven-layer device	816±42	5.1

Fig. S9 : Schematic illustration of flow channel modifications for device optimization: (a) Splitting of flow channels to arrange them in a zone of constant pressure (marked in red) between folded paper layers surrounded by staples; (b) outline of six- and seven-layer model devices (no hydrophobic walls; only green channel) used to measure the overall liquid flow-through times (mean ± 1σ for $n = 12$).

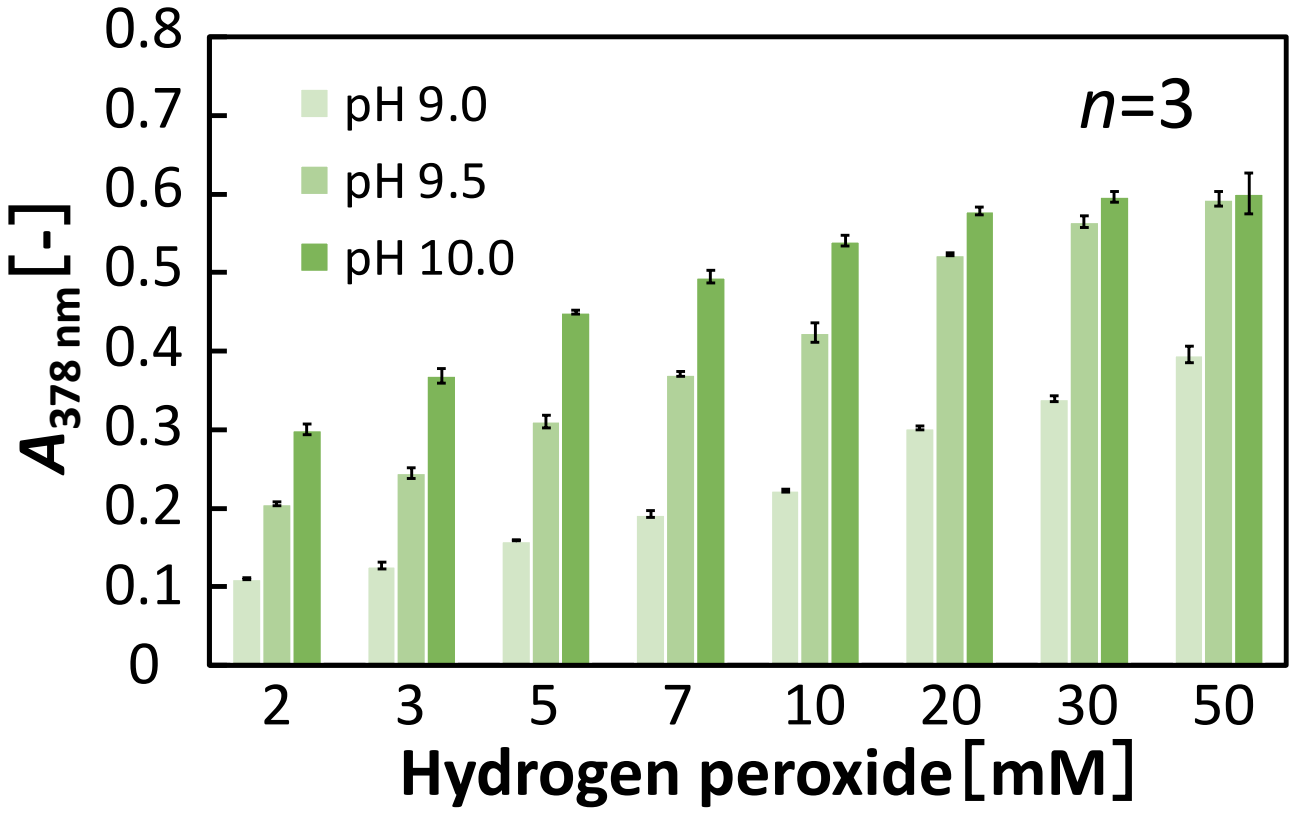


Fig. S10 : Absorbance measurements after exposing paper disks modified with boronic acid pinacol ester for 10 min to solutions of various H_2O_2 concentrations and different pH in glycine buffer (pH 9.0, 9.5, 10.0); error bars represent mean values ± 1σ (n = 3).

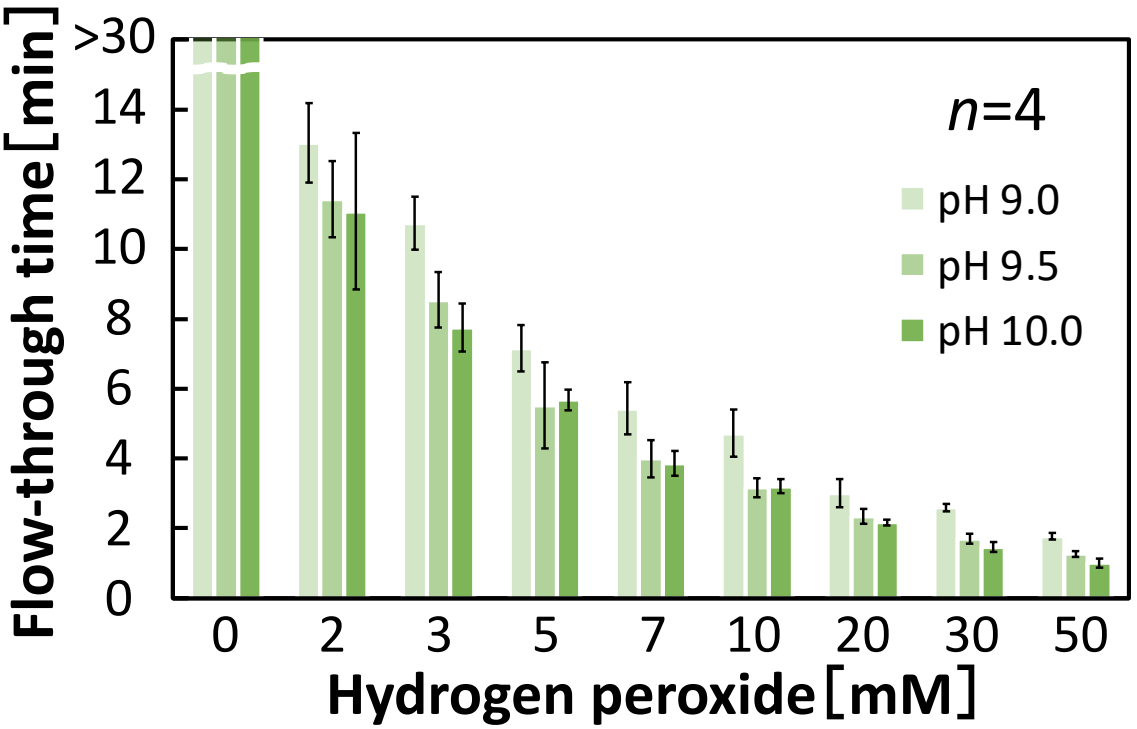


Fig. S11 : Measurement of H_2O_2 concentration-dependent flow-through times at different pH values (pH 9.0, 9.5, 10.0) using model Devices c-e (Fig. S2); error bars represent mean values ± 1σ (n = 4).

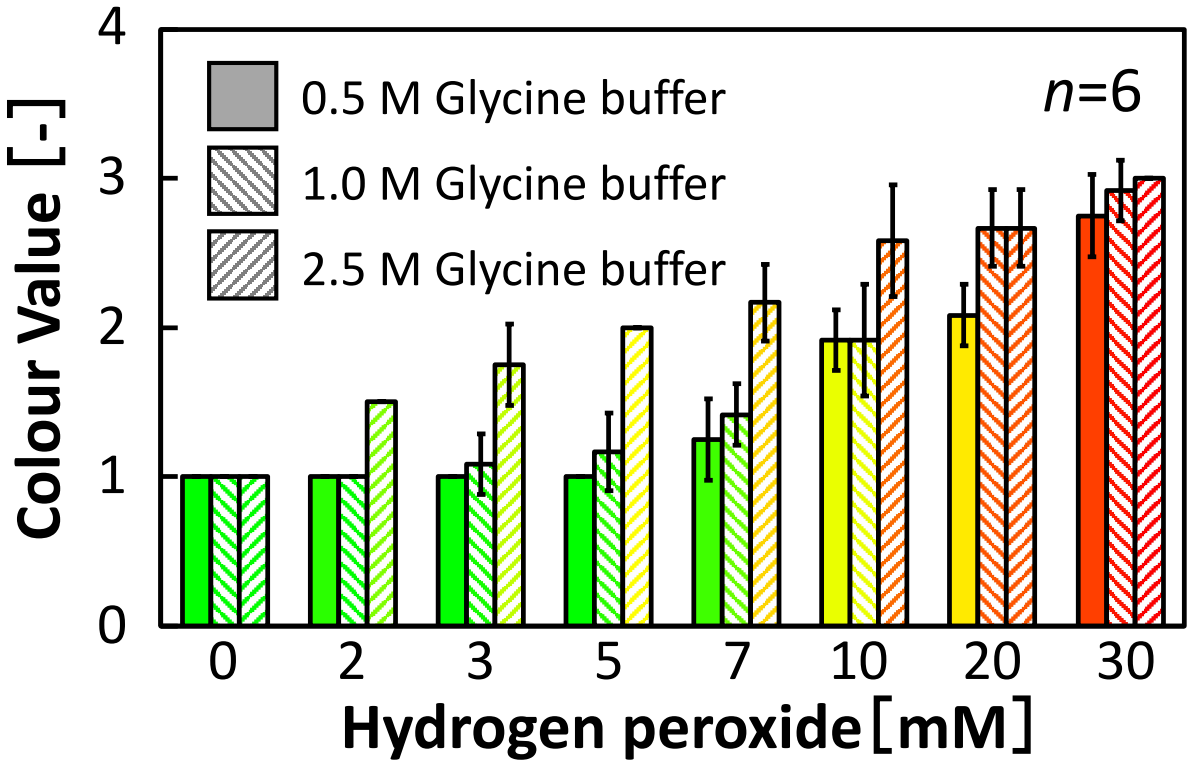


Fig. S12 : Results of traffic light assay for H_2O_2 detection demonstrating changes of the concentration threshold for displaying traffic light colours by increasing the concentration of the glycine buffer (pH 9.0) pre-deposited on the device (Device 2); error bars represent mean values ± 1σ (n = 6).

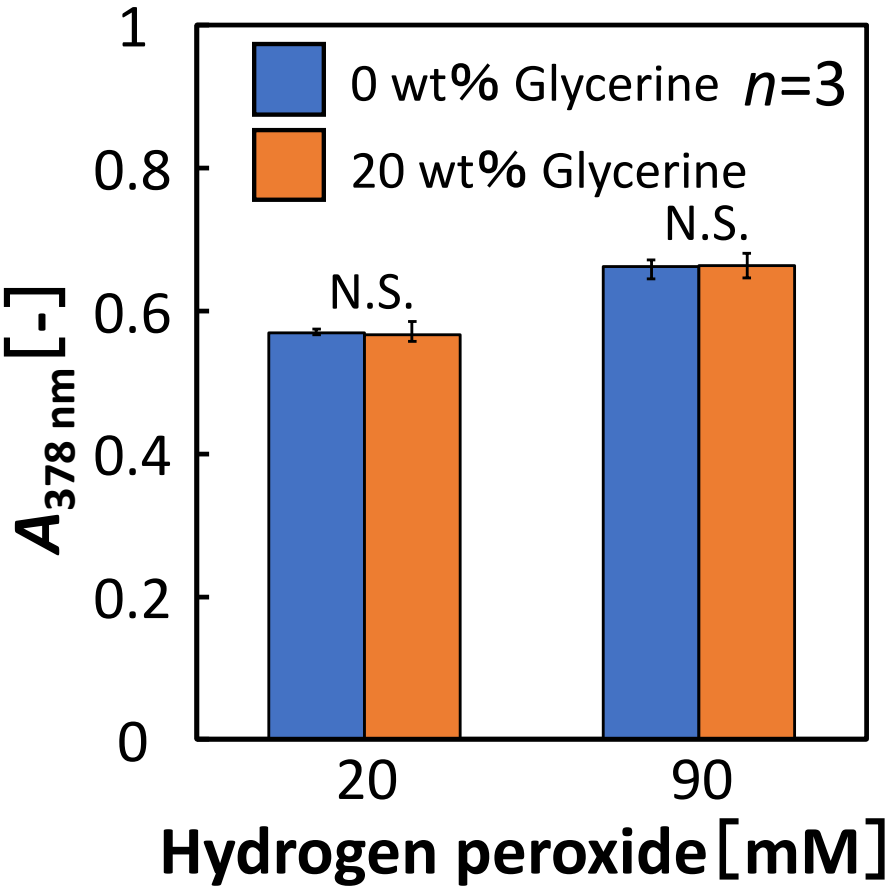


Fig. S13 : Absorbance measurements after exposing paper disks modified with boronic acid pinacol ester for 20 min to solutions of H_2O_2 in glycine buffer (pH 9.0) containing glycerine; error bars represent mean values ± 1σ (n = 3); N.S.: not significant.

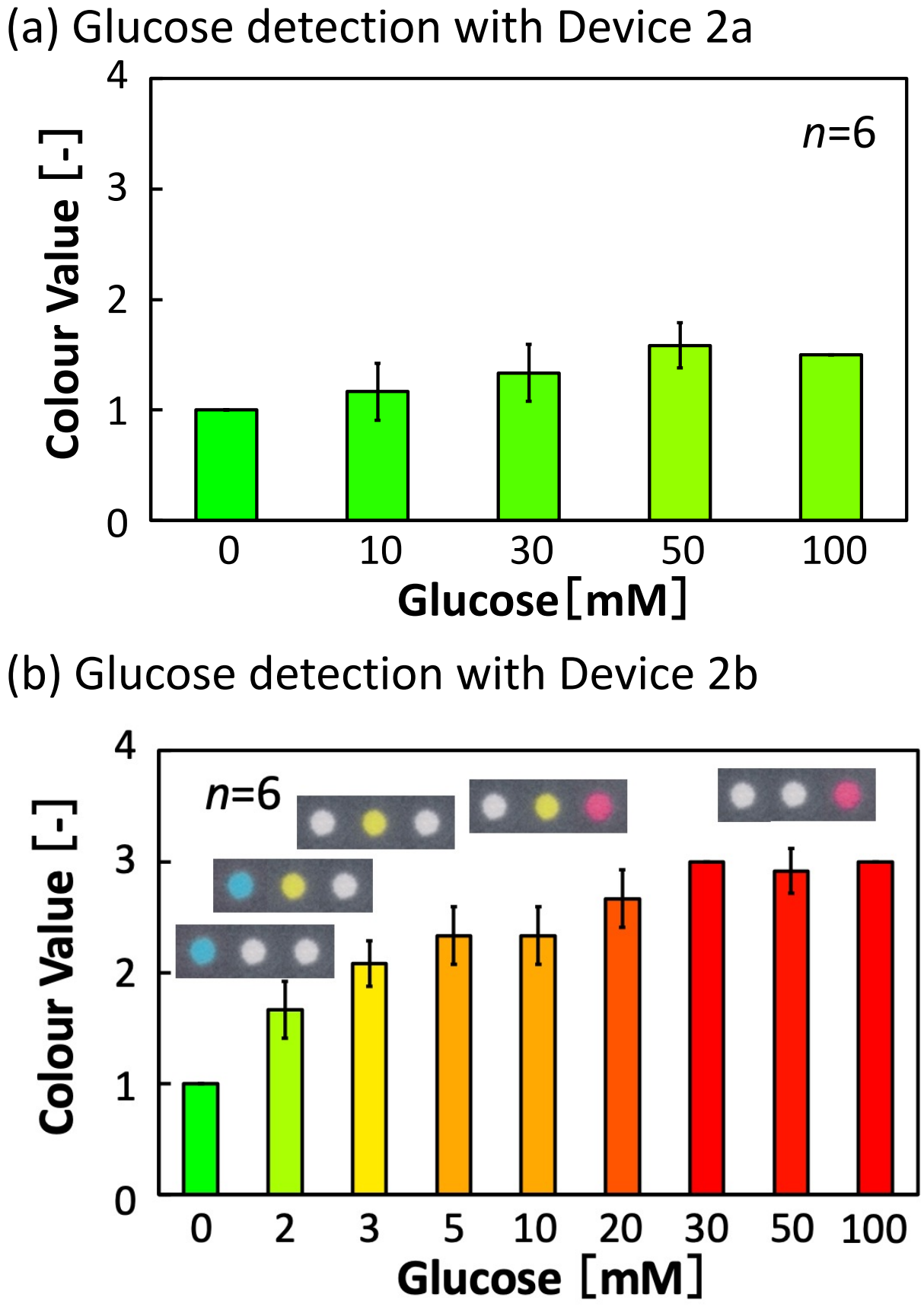


Fig. S14 : Results of traffic light colour glucose assay from aqueous samples reacted with GOx off-device: (a) Device 2a and (b) Device 2b; error bars represent mean values ± 1σ (n = 6); insets show the actually observed traffic light signal for each respective analyte concentration (note: images of colored spots are cut and pasted next to each other in magnified form; refer to Fig. S4 for an example of an actual size image).

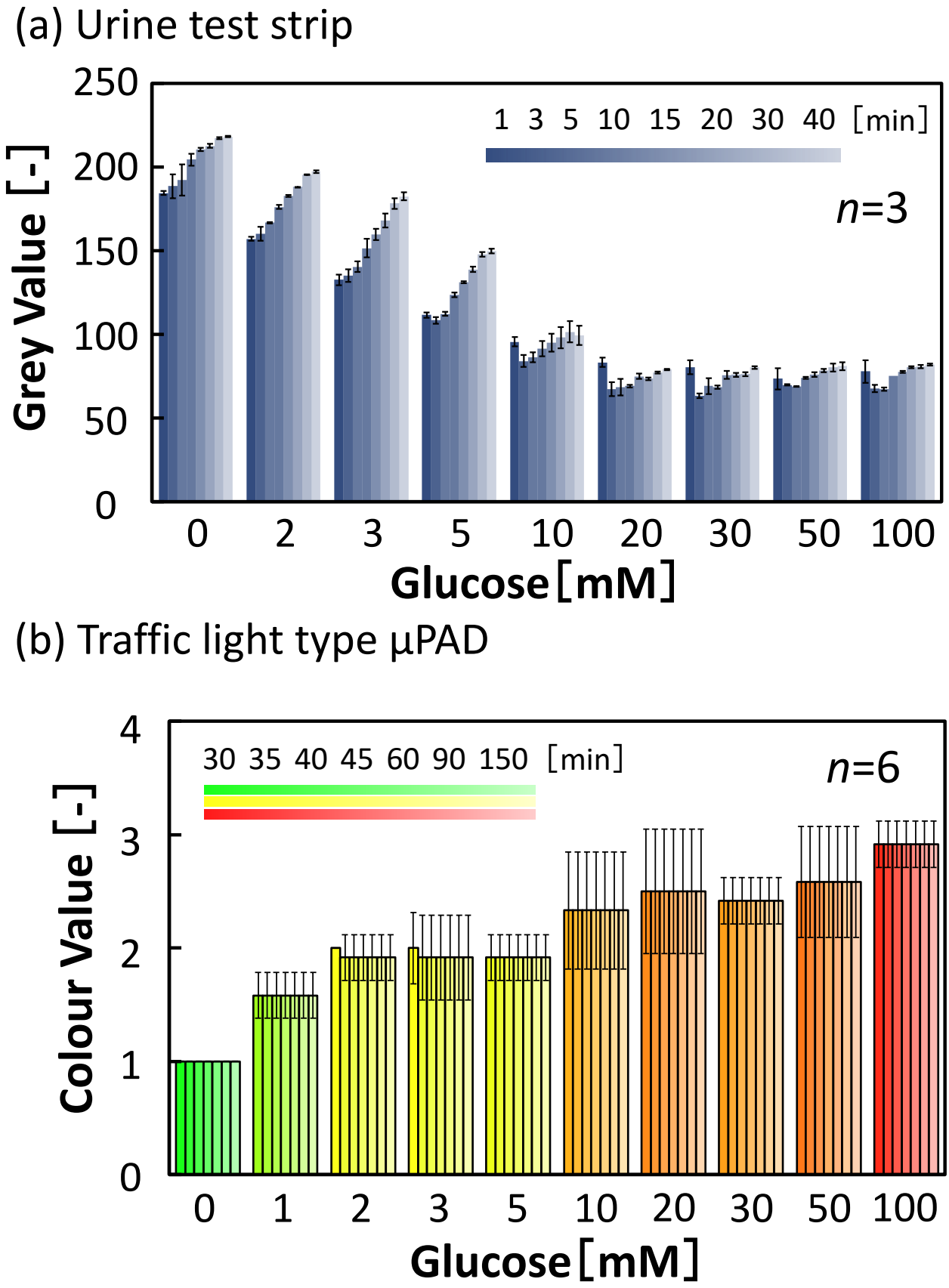


Fig. S15 : Comparison of time stability of colorimetric signals in glucose assays: (a) grey value of the glucose test zone of a urine test strip at various times from sample application (note: manufacturer indicates that colour must be read out at 1 min); error bars represent the mean values ± 1σ (n = 3); (b) traffic light colours displayed at various times from sample application; error bars represent the mean values ± 1σ (n = 6).

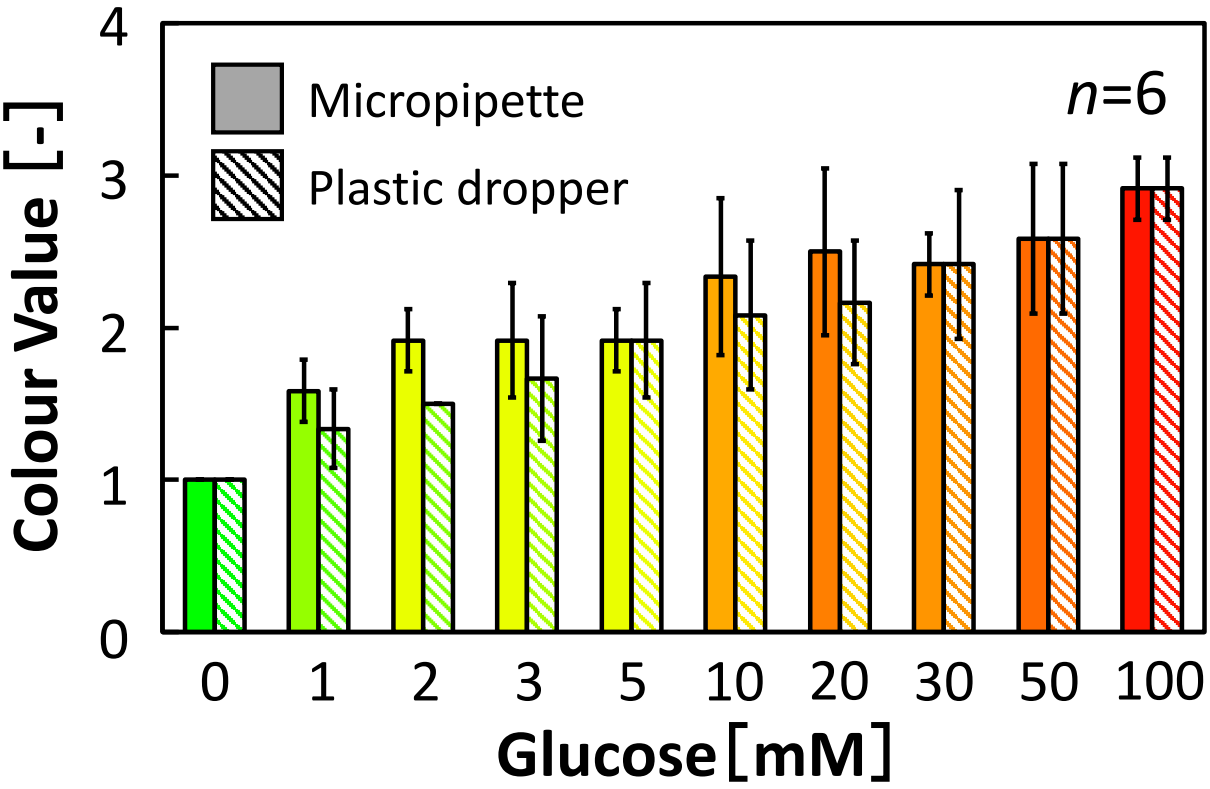


Fig. S16 : Results of traffic light assay for glucose detection using Device 3 in combination with different sample application methods (micropipette or simple plastic dropper); error bars represent the mean values ± 1σ (n = 6).

Table S1 : Composition of artificial urine matrix.

Component	Concentration［mM］
L-Lactic acid	1.1
Citric acid	2
Sodium bicarbonate	25
Urea	170
Calcium chloride	2.5
Sodium chloride	90
Magnesium sulphate	2
Sodium sulphate	10
Potassium dihydrogen phosphate	7
Di-potassium hydrogen phosphate	7
Ammonium chloride	25