## Supplementary Information

Improving quantitative control and homogeneous distribution of sample on paper-based analytical devices via drop-on-demand inkjet printing

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#### **Figures**

### FigureS1



**Fig. S1** Photos for the inside structure (A) and operational panel (B) of a commercialCanon MP288piezoelectric inkjet printer. Photos of the black cartridge (C) and the color cartridge (D) before (left) and after (right) disassembly. Black cartridge (PG-815): Key (K, Black); Color cartridge (CL-816): Cyan (C); Yellow (Y) and Magenta (M).

Figure S2





**Fig. S2** Optimization of the printed area and print quality. (A) Optical micrographs of the different printed areas with various magnification. a: 0.25 mm<sup>2</sup> with magnification of 45 times. b: 2.25 mm<sup>2</sup> with magnification of 15 times. c: 9.0 mm<sup>2</sup> with magnification of 7 times. (B) Optical micrographs of the cyan ink dots at C value range from 10 % to 100 % with different print quality. a: Standard printing. b: High-quality printing. c: Fast printing.





Swatch Options						
Swatch Name: CMYK yellow Color Type: Process Color						
Color Mode: CMYK						
Preview OK Cancel E						



**Fig. S3** Parameters setting of CMYK in the regularity research of single color. The setting of K value in the grayscale printing (A) and CMYK mode (B). The setting of C (C), M (D) and Y (E) values in the CMYK mode. (F) Optical micrographs of the black ink dots in the CMYK mode at various K values.



**Fig. S4** Parameters setting of CMYK in the regularity research of two-colors. (A) C-M pairs. (B) C-Ypairs.





d





**Fig. S5** Regularity research of two-color. Optical micrographs of M-Y pairs (a), M-C pairs (b), Y-C pairs (c) and Y-M pairs (d) showing the number change of studied color ink dots at specific opacity value with the value of interference color. Effects of (A) Y value and (B) C value on the number change of the magenta ink dots. Effects of (C) C value and (D) M value on the number change of the yellow ink dots. Error bars represent the standard deviations for three replicates.



Fig. S6 (A) Oxidation reaction catalyzed by HRP. (B) Linear relationship between color intensity and  $H_2O_2$  concentration at various K values (gray value: 10%, 30%, 50% and 100%).  $H_2O_2$  concentration: 2.5, 5, 10, 15, 25 and 50 mM. Error bars represent the standard deviations for three replicates.



**Fig. S7** Linear relationship between color intensity and  $H_2O_2$  concentration at various C values (opacity value: 10%, 30%, 50% and 100%).  $H_2O_2$  concentration: 2.5, 5, 10, 15 and 25 mM. Error bars represent the standard deviations for three replicates.

Serum	μPADs(μM)		Biochemical instrument IT3000	
samples	Mean(n=5)	RSD(%)	(µM)	
1	3.433	5.366	3.60	
2	5.571	2.337	5.11	
3	11.049	1.970	10.50	

**Table S1** Comparison of results for determination of glucose in real serum samples

 using the four ink cartridges printing approach and biochemical instrument.

 Table S2 Recovery tests of glucose in real serum samples based on four ink cartridges.

	Added	Mean value	Recovery	RSD
	(µmol)	(µmol) (n=3)	(%)	(%)
Serum samples	0	2.652	-	-
	2.5	5.391	109.60	1.405
	5	7.442	95.81	1.152
	10	13.627	109.76	0.851