

Supporting Information

Printed two-dimensional micro-zone plates for chemical analysis and ELISA

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Analytical strategy using multi-zone micro-zone plates

For the analysis of NO_2^- , a calibration series of six NO_2^- concentrations (0, 62.5, 125, 250, 500, 1000 $\mu\text{mol/L}$) was used. Four different concentrations of UA (0, 100, 500, 1000 $\mu\text{mol/L}$) were used as four levels of interferent species. Similarly, for the analysis of UA, a calibration series of six UA concentrations (0, 62.5, 125, 250, 500, 1000 $\mu\text{mol/L}$) was used. Four different concentrations of NO_2^- were used as four different levels of interferent species. One microlitre of analyte and 1 μL of interferent solutions were added to each zone for analysis. Fig. S1 and S2 show the division of a printed 96-zone plate into four regions. The division of the plate into four regions allows the six-point sample series to be analysed in four repeats, and under four different concentrations of interferent species. This combinatorial arrangement therefore allows the interference study to be carried out by this 96 (i.e., $6 \times 4 \times 4$) spot assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Region 1						Region 2					
B	NO_2^- : 0, 62.5, 125, 250, 500, 1000 μM						NO_2^- : 0, 62.5, 125, 250, 500, 1000 μM					
C	UA: 0 μM in all samples						UA: 100 μM in all samples					
D	UA: 0 μM in all samples						UA: 100 μM in all samples					
E	Region 3						Region 4					
F	NO_2^- : 0, 62.5, 125, 250, 500, 1000 μM						NO_2^- : 0, 62.5, 125, 250, 500, 1000 μM					
G	UA: 500 μM in all samples						UA: 1000 μM in all samples					
H	UA: 500 μM in all samples						UA: 1000 μM in all samples					

Fig. S1 A printed 96-zone plate was divided into four regions to perform NO_2^- assays under four levels of UA concentrations as interferent species.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Region 1						Region 2					
B	UA: 0, 62.5, 125, 250, 500, 1000 μM						UA: 0, 62.5, 125, 250, 500, 1000 μM					
C	NO_2^- : 0 μM in all samples						NO_2^- : 100 μM in all samples					
D	NO_2^- : 0 μM in all samples						NO_2^- : 100 μM in all samples					
E	Region 3						Region 4					
F	UA: 0, 62.5, 125, 250, 500, 1000 μM						UA: 0, 62.5, 125, 250, 500, 1000 μM					
G	NO_2^- : 500 μM in all samples						NO_2^- : 1000 μM in all samples					
H	NO_2^- : 500 μM in all samples						NO_2^- : 1000 μM in all samples					

Fig. S2 A printed 96-zone plate was divided into four regions to perform UA assays under four levels of NO_2^- concentrations as interferent species.

Protocol for the direct ELISA of ferritin

The following four-step protocol for the direct ELISA of ferritin was followed in our study.

Step 1: add 4 μL of five ferritin solutions of different concentrations into five zones and incubated until dry.



Step 2: dip into a 1% BSA blocking buffer and incubated for 20 min.



Step 3: wash twice with PBS solution, then add 2 μL of 2 mg/mL AB7334 into each zone and incubated for 20 min.



Step 4: wash 3 times with PBS solution, then add 2 μL TMB substrate into each zone and incubated for 2 min in dark.



Fig. S3 A schematic of the protocol of direct ferritin ELISA on 2D micro-zone plates

Liquid handling capacity of printed micro-zones

Fig. S4 shows the addition of different volumes of ink jet inks into the printed cellulose powder micro-zones. From columns 1 to 9, the ink volume starts from 0.1 μL , with increments of 0.1 μL . From columns 10 to 12, the ink volume starts from 1 μL , with increments of 1 μL . The micro-zones cannot be completely filled by 0.1 μL of ink, but can be filled by all inks by around 0.4 μL of ink. Since the zones are porous, they have higher actual surface area and therefore higher apparent surface energy than the surrounding polymer film. Based on Wenzel's model,^[12,13] if a surface is hydrophilic, a liquid will wet the porous and rough area where the actual surface area is higher. This feature of the printed cellulose powder plate is used for liquid sample control, since liquid does not have the tendency to "leak" out of the printed micro-zone.

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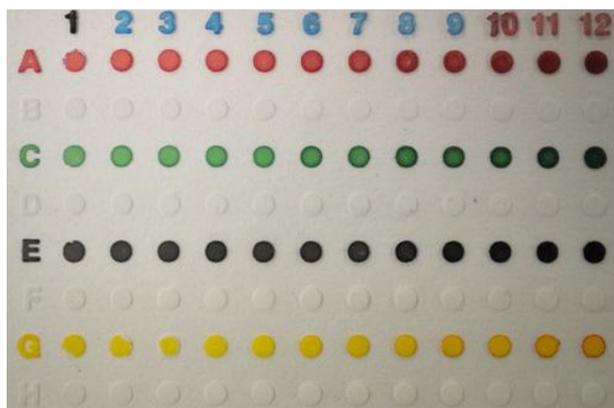


Fig. S4 A photo showing the addition of different volumes of ink jet inks to micro-zones: From columns 1 to 9, the ink volume starts from 0.1 μL , with increments of 0.1 μL . From columns 10 to 12, the ink volume starts from 1 μL , with increments of 1 μL .

Colour display ability of the printed micro-zone plate

Fig. S5 (a) shows the colour display ability of a printed cellulose multi-zone micro-zone plate. One microlitre of ink solution was introduced into each of the microzones using a micropipette. Fig. S5 (b) shows the colour density measurements of the micro-zones measured with the X-Rite densitometer.

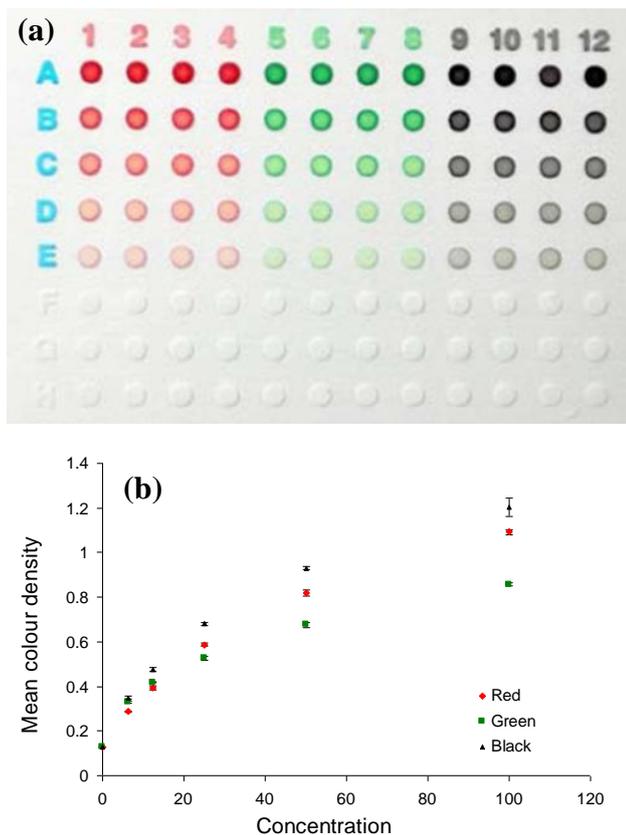


Fig. S5 (a) A photo of a micro-zone plate with serially diluted Canon ink jet inks added into the micro zones: 0 \times (100%, undiluted), 2 \times (50%, two fold dilution), 4 \times , 8 \times and 16 \times . The volume of inks added to each zone was 0.3 μL . (b) Plot of measured colour density using the X-rite colour densitometer against ink concentration.

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The plot of colour density against ink concentration gives a non-linear calibration curve; at very low concentration the calibration curves are nearly linear, but the slopes of the curves decrease when the ink concentration becomes higher.

25 This trend is similar to that of the paper sensors.^[14] Visually the colour density of the four repeats of each ink concentration is consistent; this is also reflected in the small measurement error bars, which are the standard deviation of the four measurements. The colour density of the empty
30 micro-zone is the reflective optical density of the printed zone of cellulose powder.

References

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40