Electronic Supplementary Information

# All-Printed Semiquantitative Paper-Based Analytical Devices Relying on QR Code Array Readout

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**Abbreviations**

URL: Universal Resource Locator

MES: 2-(N-morpholino)ethanesulfonic acid

NaOH: Sodium hydroxide

Zincon: (1-(2-Hydroxycarbonylphenyl)-5-(2-hydroxy-5-sulfophenyl)-3phenylformazan, sodium salt)

Amaranth: Trisodium 2-hydroxy-l-(4-sulfonato-1-naphthylazo)naphthalene 3,6-disulfonate

PAH: Poly(allyl amine chloride)

PDDA: Poly(diallyl ammonium chloride)

## Reagents

Ultrapure water (>18 MΩ cm) was obtained from a Direct-Q® 3UV ultrapure water purification system (MilliporeSigma, Burlington, MA) and used for the preparation of all aqueous solutions. Zincon (1-(2-hydroxycarbonylphenyl)-5-(2-hydroxy-5-sulfophenyl)-3phenylformazan, sodium salt) and 2-(N-morpholino)ethanesulfonic acid (MES) were purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). Sodium hydroxide (NaOH) and copper (II) chloride were purchased from FUJIFILM Wako Pure Chemical Industries (Osaka, Japan). Poly(diallyl ammonium chloride) (PDDA), poly(allyl amine chloride) (PAH) (molecular weight of 17500 g mol-1) and trisodium 2-hydroxy-l-(4-sulfonato-1-naphthylazo)naphthalene 3,6-disulfonate (Amaranth) were obtained from MilliporeSigma (Burlington, MA).

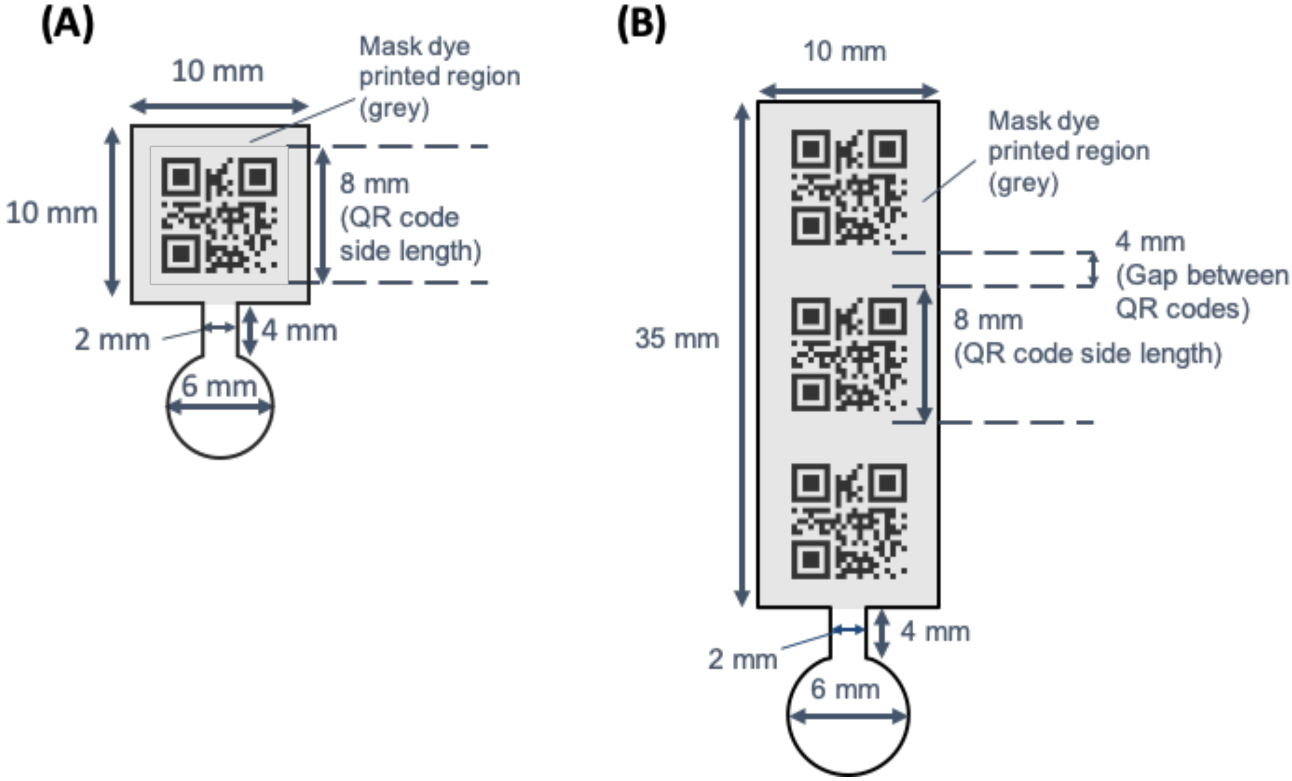


**Fig. S-1.** Schematic illustration of device dimensions: (A) Design A; (B) Design B.

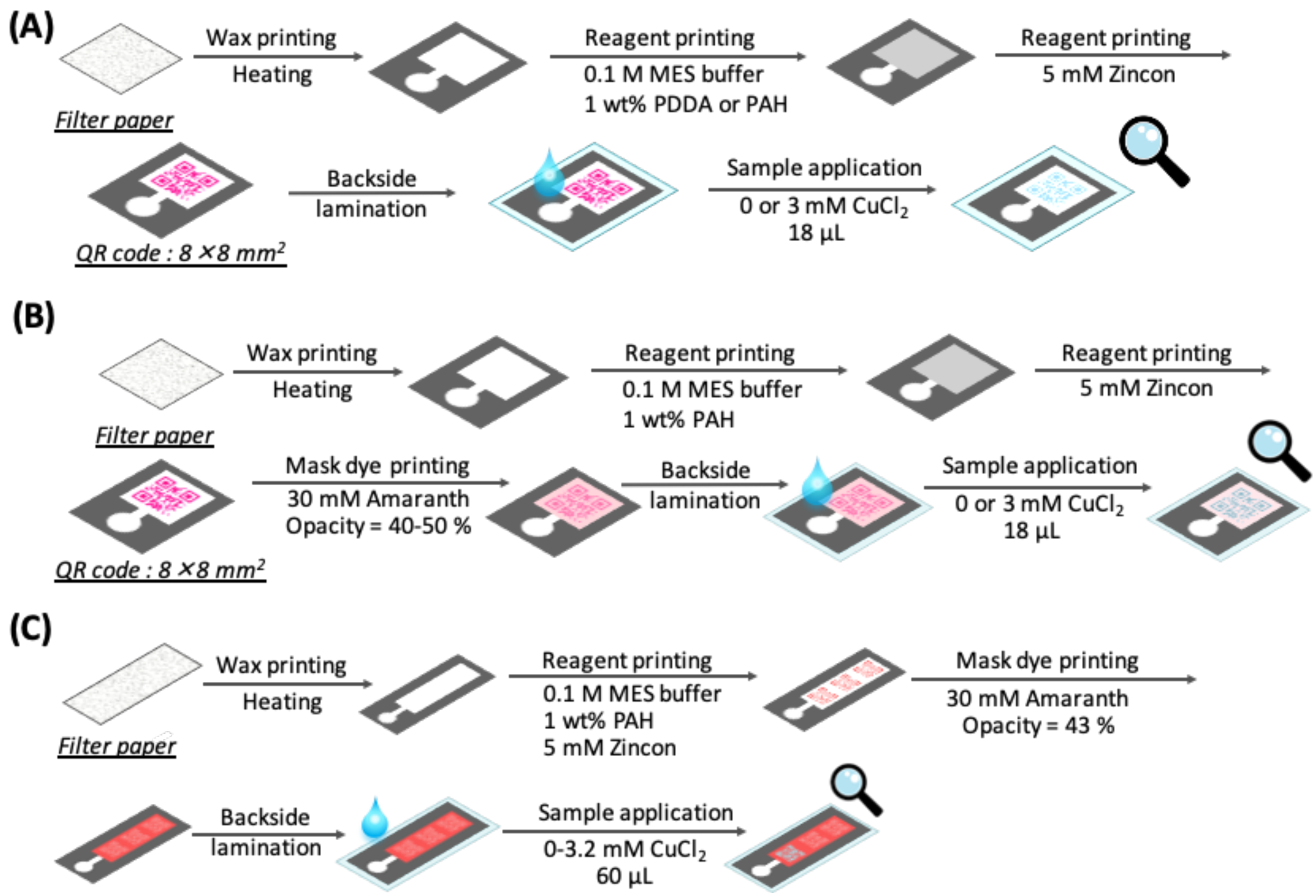


**Fig. S-2.** Overview of the entire PAD fabrication process: (A) Design A for evaluation of immobilisation capability; (B) Design A for evaluation of mask function; (C) Design B.

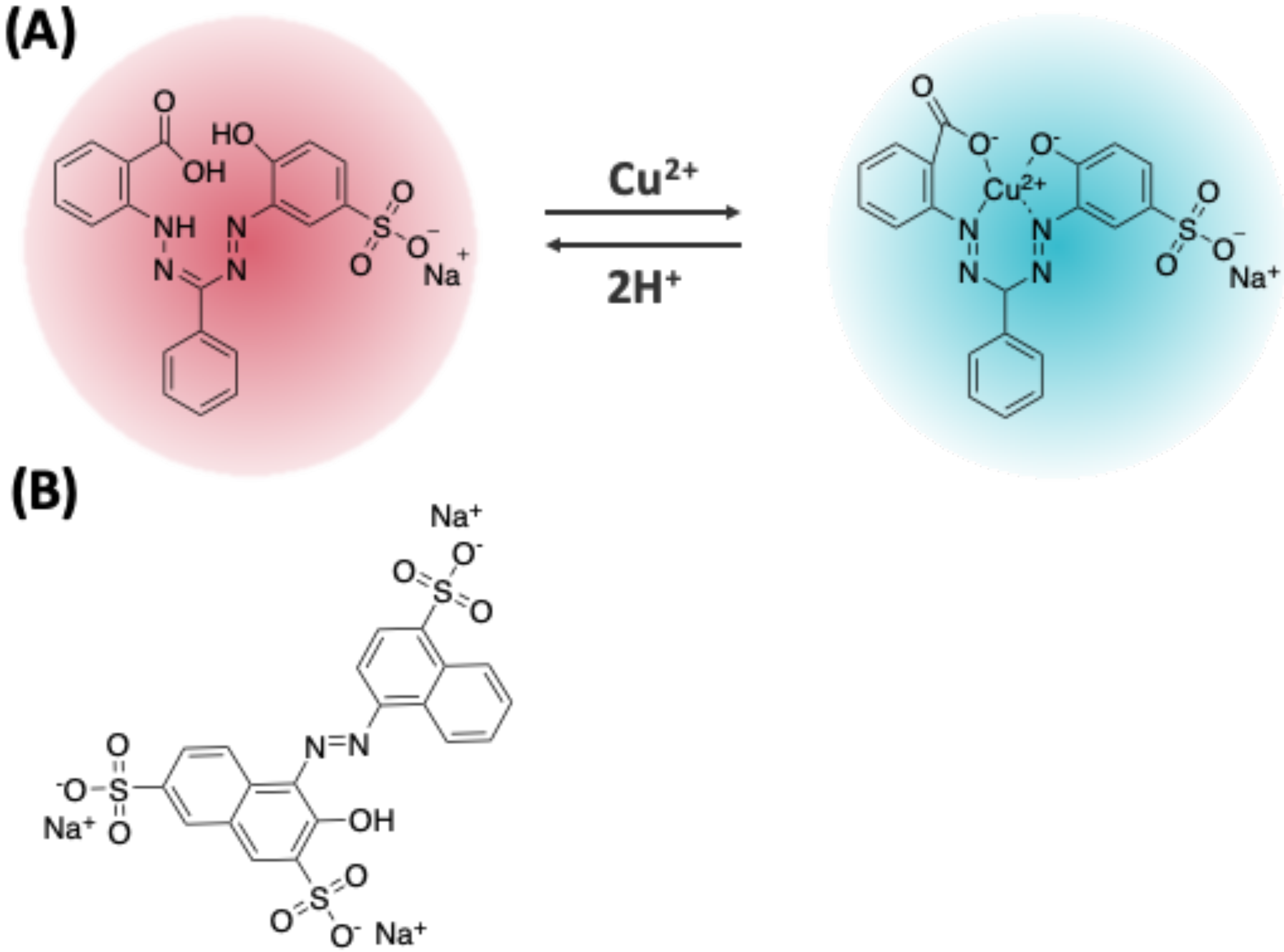


**Fig. S-3.** (A) Structure of Zincon and its $Cu^{2+}$ detection chemistry; (B) Structure of Amaranth.

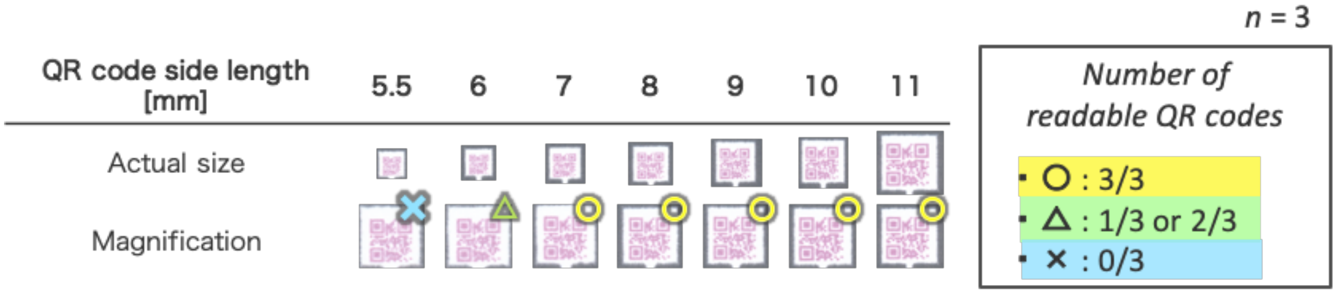


**Fig. S-4.** Influence of QR code dimensions on readout ability by the barcode reader app due to print resolution limitation. QR code patterns were printed in two print cycles with Zincon ink, after the deposition of MES buffer and PAH ink onto the entire region.

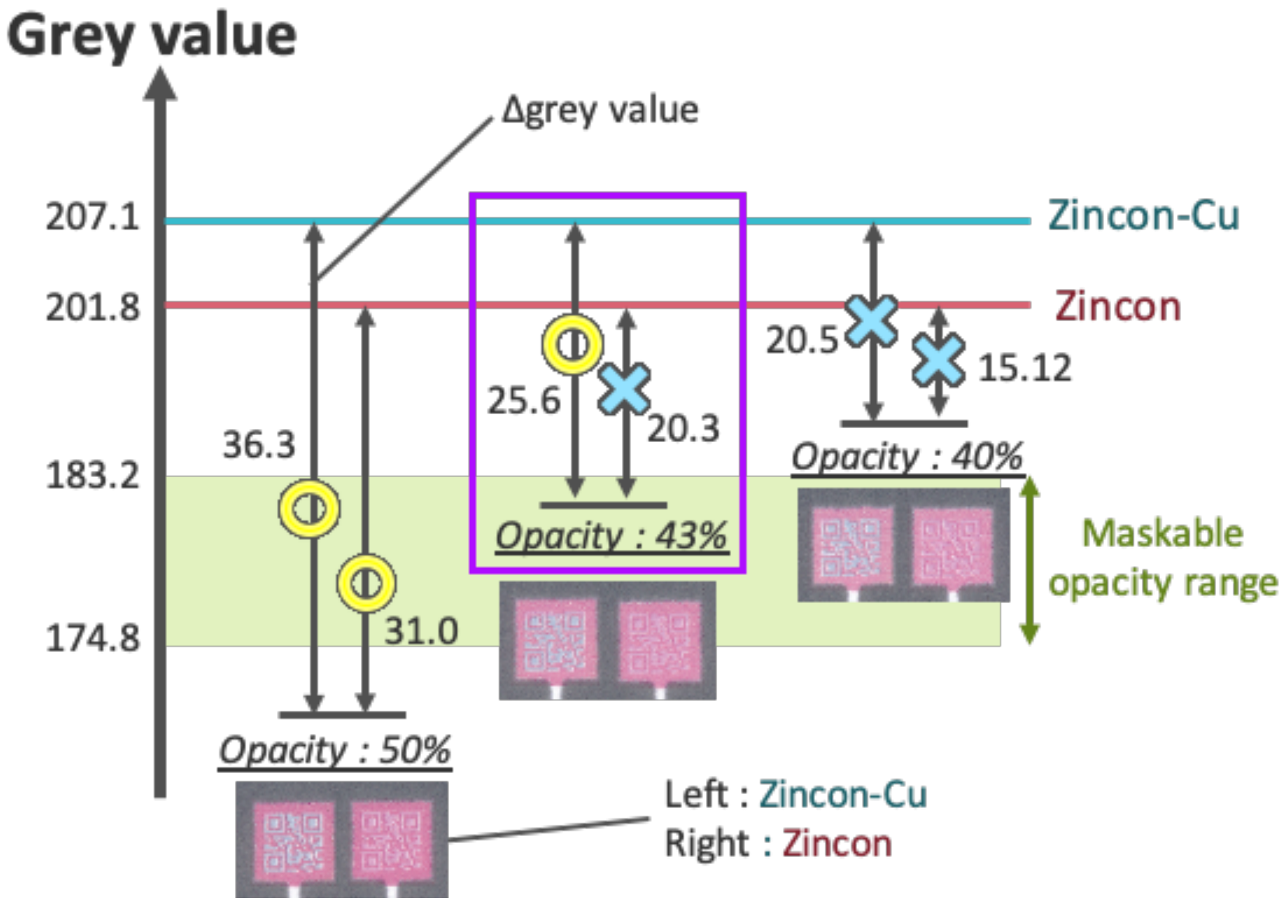


**Fig. S-5.** Illustration of mask principle: The optimal print opacity range was determined by the relationship between the Δgrey values of the free Zincon state and the mask, and between the $Cu^{2+}$-Zincon complexed state and the mask. The ideal condition is with the QR code being readable (O) for the $Cu^{2+}$-Zincon complexed state and non-readable (×) for the free Zincon state, which has been achieved within the green highlighted range; black arrows and numbers indicate Δgrey values.

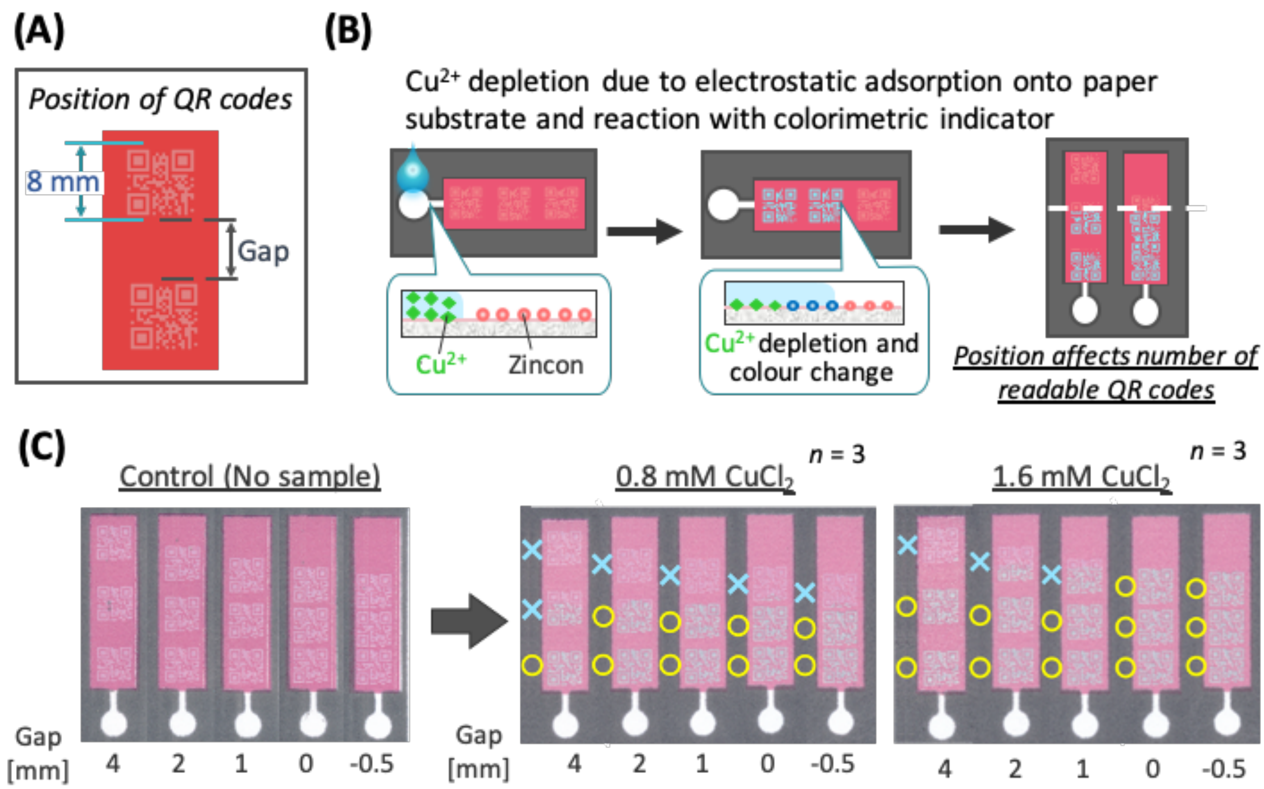


**Fig. S-6.** Influence of gaps between QR codes in an array: (A) Illustration of QR code gap size; (B) Schematic illustration of QR code gap influence on number of readable QR codes; (C) Photograph of experimental results illustrating changes in number of readable QR codes by varying gaps from -0.5 to 4 mm; all experiments performed in triplicate.

**Table S-1.** $Cu^{2+}$ concentration-dependent grey values for different regions of devices according to Design B (n=3).

| $CuCl_2$ [mM] | Grey value after sample application | | |
|---|---|---|---|
| | Unreacted areas (Zincon QR code pattern) | Reacted areas (Zincon QR code pattern) | Amaranth mask region |
| 0 | 202.5 ± 0.3 | - | 182.5 ± 0.2 |
| 0.1 | 203.1 ± 0.4 | 208.6 ± 0.1 | 181.4 ± 0.3 |
| 0.4 | 202.4 ± 0.2 | 208.5 ± 0.6 | 181.3 ± 0.3 |
| 0.8 | 203.1 ± 0.4 | 208.3 ± 0.4 | 181.7 ± 0.2 |
| 1.6 | 202.8 ± 0.6 | 208.4 ± 0.3 | 181.3 ± 0.2 |
| 2 | 203.1 ± 0.5 | 208.5 ± 0.2 | 181.8 ± 0.1 |
| 3.2 | - | 208.5 ± 0.2 | 181.6 ± 0.1 |

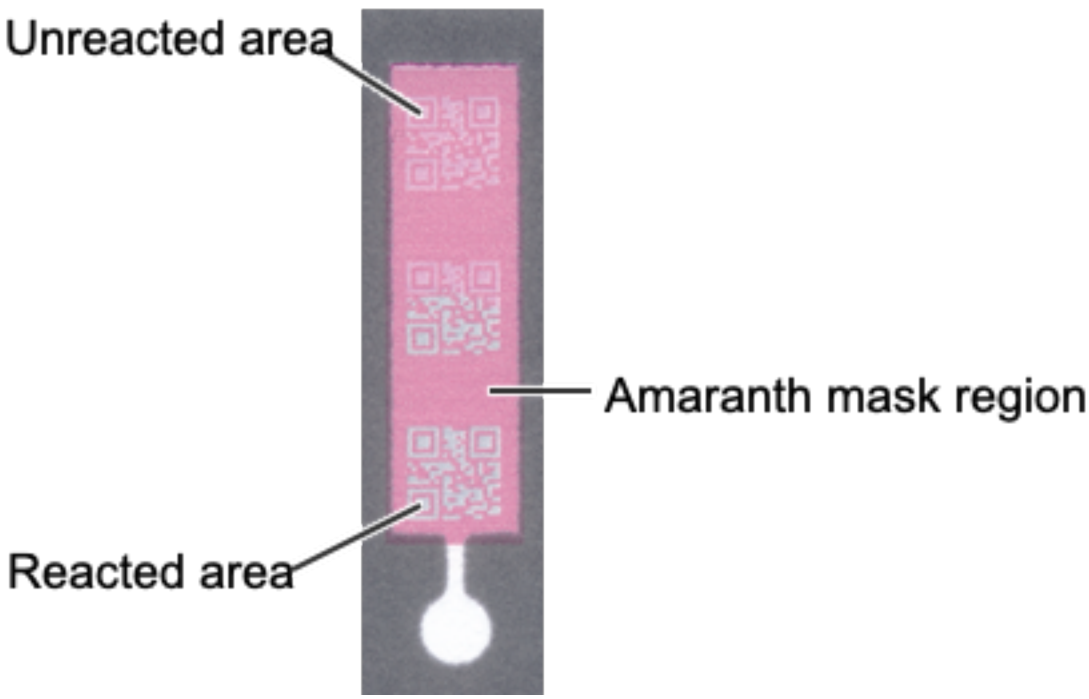

**Table S-2.** Optimization of mask condition (n=3).

| Opacity [%] | Grey value | No sample | 0 mM $CuCl_2$ | 3 mM $CuCl_2$ |
|---|---|---|---|---|
| 40 | 186.6 ± 0.2 | × (0/3) | × (0/3) | × (0/3) |
| 41 | 184.7 ± 0.5 | × (0/3) | × (0/3) | △ (1/3) |
| 42 | 183.2 ± 0.3 | × (0/3) | × (0/3) | O (3/3) |
| 43 | 181.5 ± 0.3 | × (0/3) | × (0/3) | O (3/3) |
| 44 | 179.7 ± 0.5 | × (0/3) | × (0/3) | O (3/3) |
| 45 | 177.7 ± 0.3 | × (0/3) | × (0/3) | O (3/3) |
| 46 | 176.8 ± 0.4 | × (0/3) | × (0/3) | O (3/3) |
| 47 | 174.8 ± 0.3 | × (0/3) | × (0/3) | O (3/3) |
| 48 | 173.7 ± 0.3 | △ (2/3) | △ (1/3) | O (3/3) |
| 49 | 172.3 ± 0.5 | O (3/3) | O (3/3) | O (3/3) |
| 50 | 170.8 ± 0.5 | O (3/3) | O (3/3) | O (3/3) |

O All QR codes of the three replicated devices recognised; △ one or two of them recognised;
× none of them recognised

The green shaded area indicates the optimal condition, with all QR codes being masked before sample application or after application of a blank sample, becoming recognisable for barcode reader app after $Cu^{2+}$ sample application.

**Table S-3.** Ideal readout result for $Cu^{2+}$ detection.

| Specification | QR code 1[a)] Low conc. range detection | QR code 2[b)] Mid conc. range detection | QR code 3[c)] High conc. range detection |
|---|---|---|---|
| No sample | × | × | × |
| Blank sample | × | × | × |
| Low conc. sample | readable | × | × |
| Mid conc. sample | readable | readable | × |
| High conc. sample | readable | readable | readable |

[a)] Located closest to sample inlet

[b)] Centre position

[c)] Located furthest downstream of sample flow

**Table S-4.** Influence of types of smartphone (n=3).

| $CuCl_2$ [mM] | Number of readable QR codes | |
|---|---|---|
| | Xperia | iPhone |
| 0 | 0/3 | 0/3 |
| 0.1 | 0/3 | 0/3 |
| 0.4 | 1/3 | 1/3 |
| 0.8 | 1/3 | 1/3 |
| 1.6 | 2/3 | 2/3 |
| 2 | 2/3 | 2/3 |
| 3.2 | 3/3 | 3/3 |

**Table S-5.** Influence of environmental lighting (n=3).

| $CuCl_2$ [mM] | Number of readable QR codes | | |
|---|---|---|---|
| | 1303 lux | 25 lux | 1.2 lux |
| 0 | 0/3 | 0/3 | 0/3 |
| 0.1 | 0/3 | 0/3 | 0/3 |
| 0.4 | 1/3 | 1/3 | 1/3 |
| 0.8 | 1/3 | 1/3 | 1/3 |
| 1.6 | 2/3 | 2/3 | 2/3 |
| 2 | 2/3 | 2/3 | 2/3 |
| 3.2 | 3/3 | 3/3 | 3/3 |