Electronic Supplementary Material (ESI) for Analyst.


Supplemental Information for

# Fully inkjet-printed distance-based paper microfluidic devices for colorimetric calcium determination using ion-selective optodes

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**Preparation of ion-selective optode nanosphere (nano-optode) suspension**

4.28 mg of NaTFPB (sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate), 1.68 mg of CH1 (chromoionophore I), 3.2 mg of DOS (bis(2-ethylhexyl) sebacate), 3.0 mg of F127 (Pluronic® F-127) and 3.90 mg of calcium ionophore IV were dissolved in 1.2 mL of THF to obtain a homogeneous solution. 1.0 mL of this prepared cocktail was injected into 4.5 mL of deionized water on a vortex with a spinning speed of 1000 r/min, followed by organic solvent removal with a stream of $N_2$ gas for 40 min. The suspension of nano-optodes contained nanosphere particles of approximately 200 nm in diameter with a polydispersity index (PDI) of 0.273. This solution was directly used for inkjet printing without any further processing.

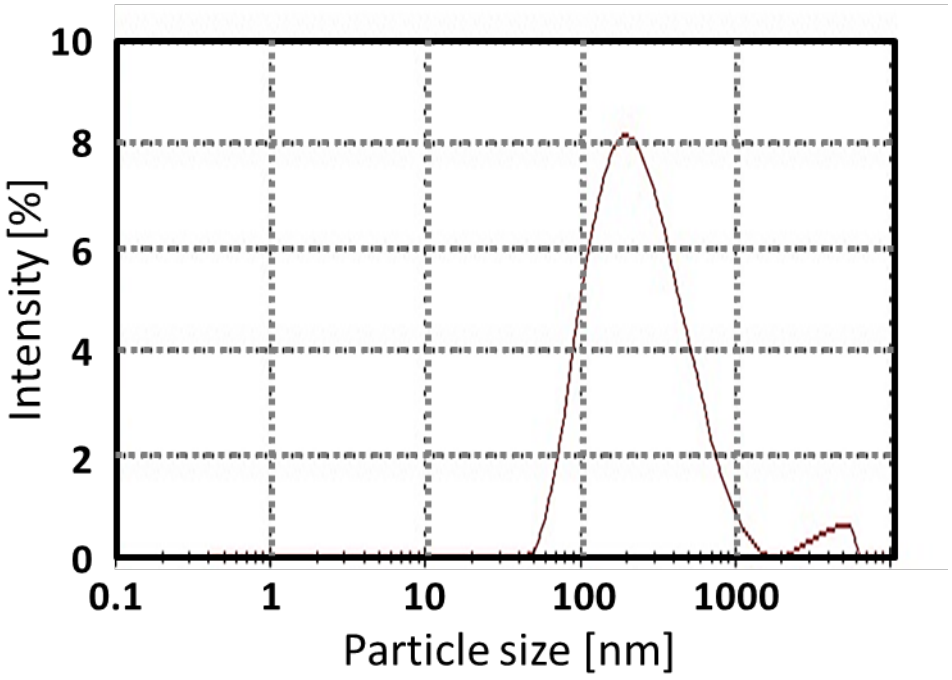


**Figure S1.** Hydrodynamic diameter of the prepared nano-optodes measured by dynamic light scattering (DLS).

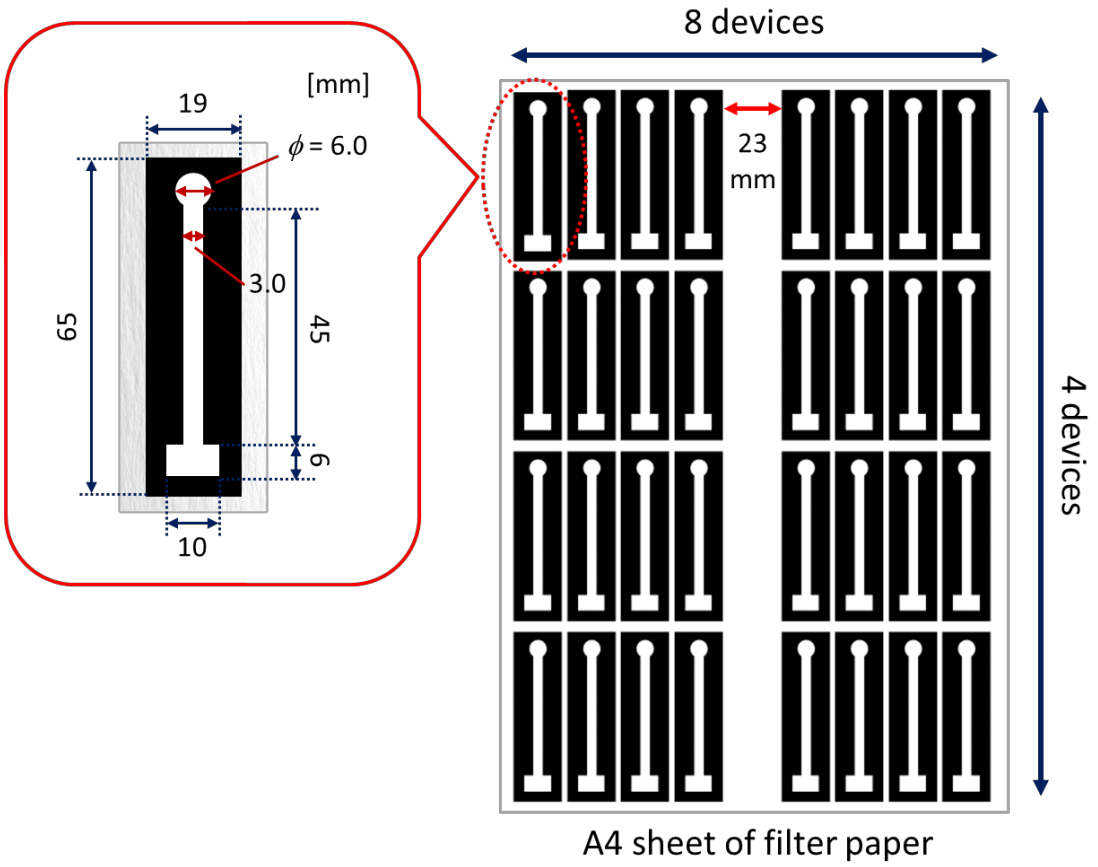


**Figure S2.** Schematic illustration of the wax barrier patterns printed on an A4-size filter paper sheet. The dimensions in the red box represent the settings in the PowerPoint graphic software.

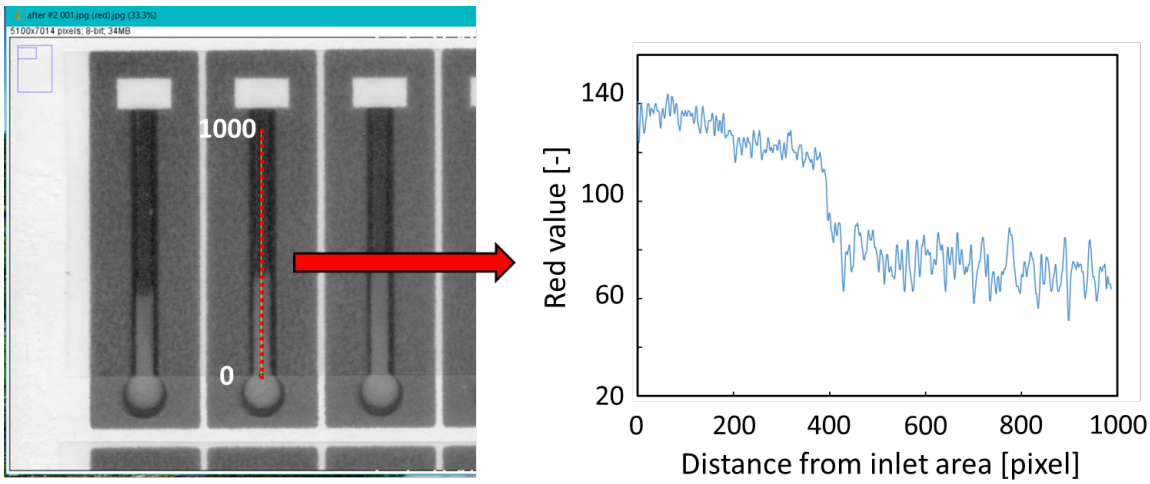


**Figure S3.** Procedure for obtaining colorimetric response profiles along a detection channel of colour-developed μPADs; after extraction of the red colour channel from a scanned image, the "smoothing" function of Image J was applied.

**Experimental procedure of complexometric titration for quantifying the amounts of $Ca^{2+}$ in drinking or tap waters**

Approximately 5 mL of 8 N KOH was added into 50 mL of the water sample (≈ pH 13), followed by stirring for several minutes. Next, approximately 0.1 mg of NN (2-hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid) indicator was added into the pretreated sample solution and titrated with 0.01 N EDTA solution ($f$ = 1.001). The endpoint of the complexometric titration was defined as the required volume of EDTA solution to obtain a colorimetric change of the NN indicator from dark red to blue.

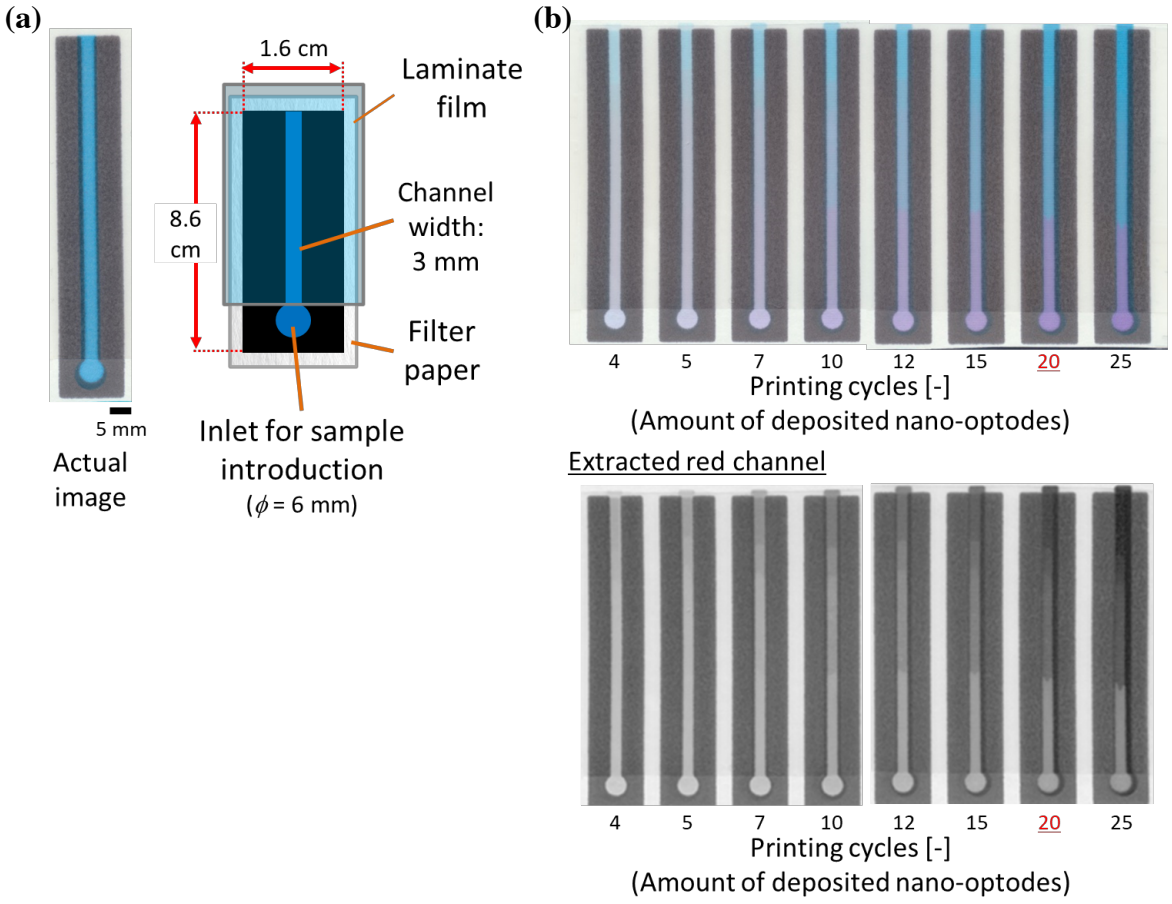


**Figure S4.** (a) Design of the developed distance-based µPADs for evaluation of the amount of nano-optodes: actual scanned image of a µPAD (left) and the corresponding dimensions (right). (b) Scanned images of distance-based µPADs with different amounts of printed nano-optodes (number of printing cycles: 4, 5, 7, 10, 12, 15, 20 and 25) 45 min after application of 30 µL of pH-buffered (50 mmol $L^{-1}$ HEPES-TMAOH buffer pH 7.0) 1.0 mmol $L^{-1}$ $Ca^{2+}$ solution.

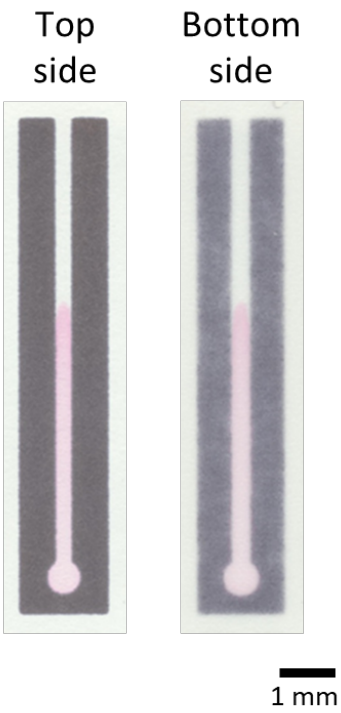


**Figure S5.** Evaluation of wax barrier resistance against the presence of surfactants: 30 μL of aqueous solution containing a food dye (0.02wt% acid red) was applied to an unlaminated μPAD containing surfactant (F-127) micelles printed from a magenta cartridge at 20 cycles to mimic the conditions found in $Ca^{2+}$-selective distance-based μPADs; the F-127 micelle ink suspension was prepared according to the same procedure as described for the preparation of $Ca^{2+}$-selective nano-optodes, however without the addition of sensing reagents (ionophore, chromoionophore, ion-exchanger).

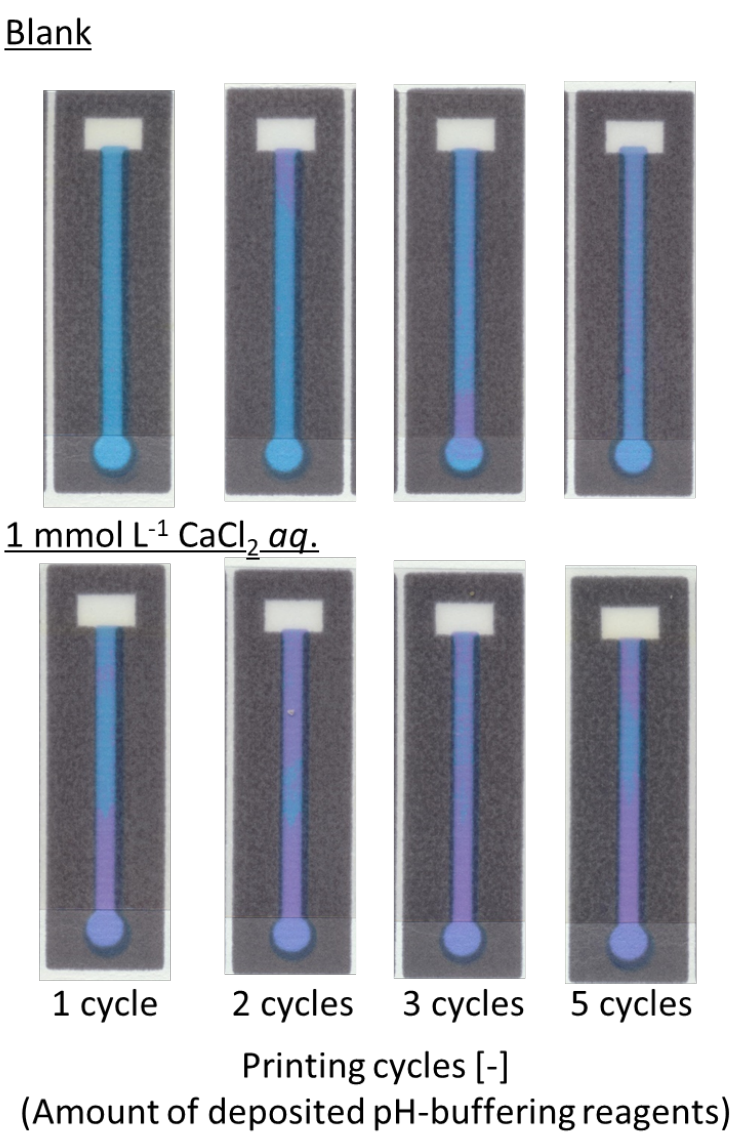


**Figure S6.** Evaluation of amounts of pH-buffering reagents (250 mmol $L^{-1}$ HEPES-TMAOH buffer pH 7.0) printed onto the inlet areas and the flow channels of μPADs (printing cycles of pH-buffering reagents: 1, 2, 3, 5 cycles); 30 μL of blank (pure $H_2O$) or 1 mmol $L^{-1}$ aqueous $CaCl_2$ solution was applied onto a μPAD.

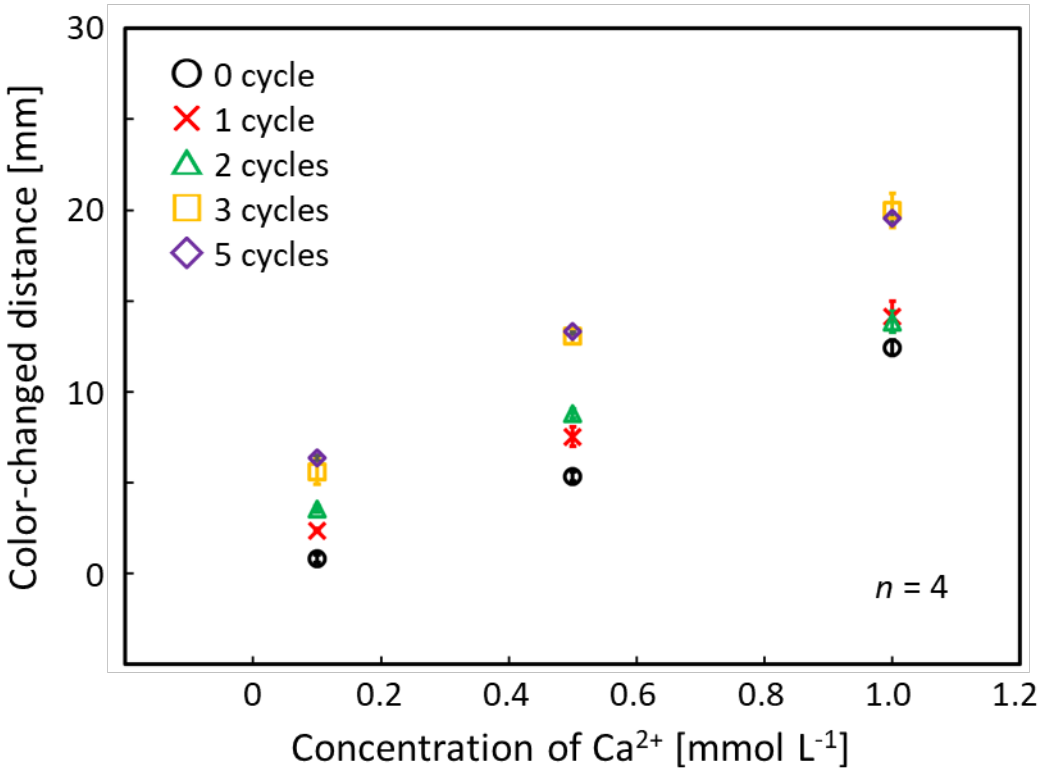


**Figure S7.** Evaluation of the amounts of $MgCl_2$ printed onto the inlet area of μPADs (printing cycles of $MgCl_2$: 0, 1, 3, 5 cycles); each data point has been obtained by measurements with 4 individual single-use distance-based μPADs; 30 μL of aqueous $CaCl_2$ solution was applied onto a μPAD; error bars indicate the standard deviations; incubation time: 45 min.

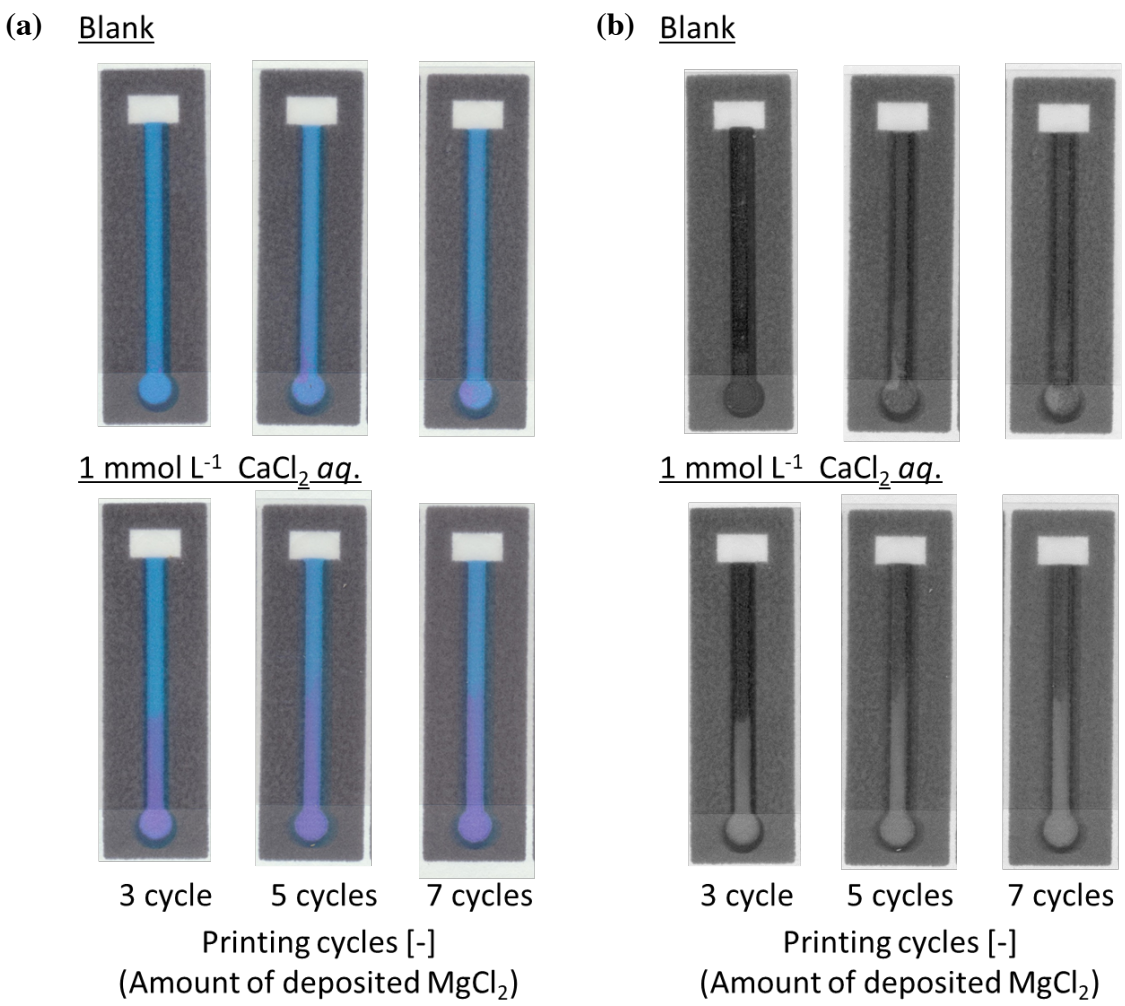


**Figure S8.** (a) Scanned images and (b) extracted red channel of distance-based μPADs with different amounts of printed $MgCl_2$ (number of printing cycles: 3, 5, 7) 45 min after application of 30 μL of blank sample (water) and 1.0 mmol $L^{-1}$ $Ca^{2+}$ solution.

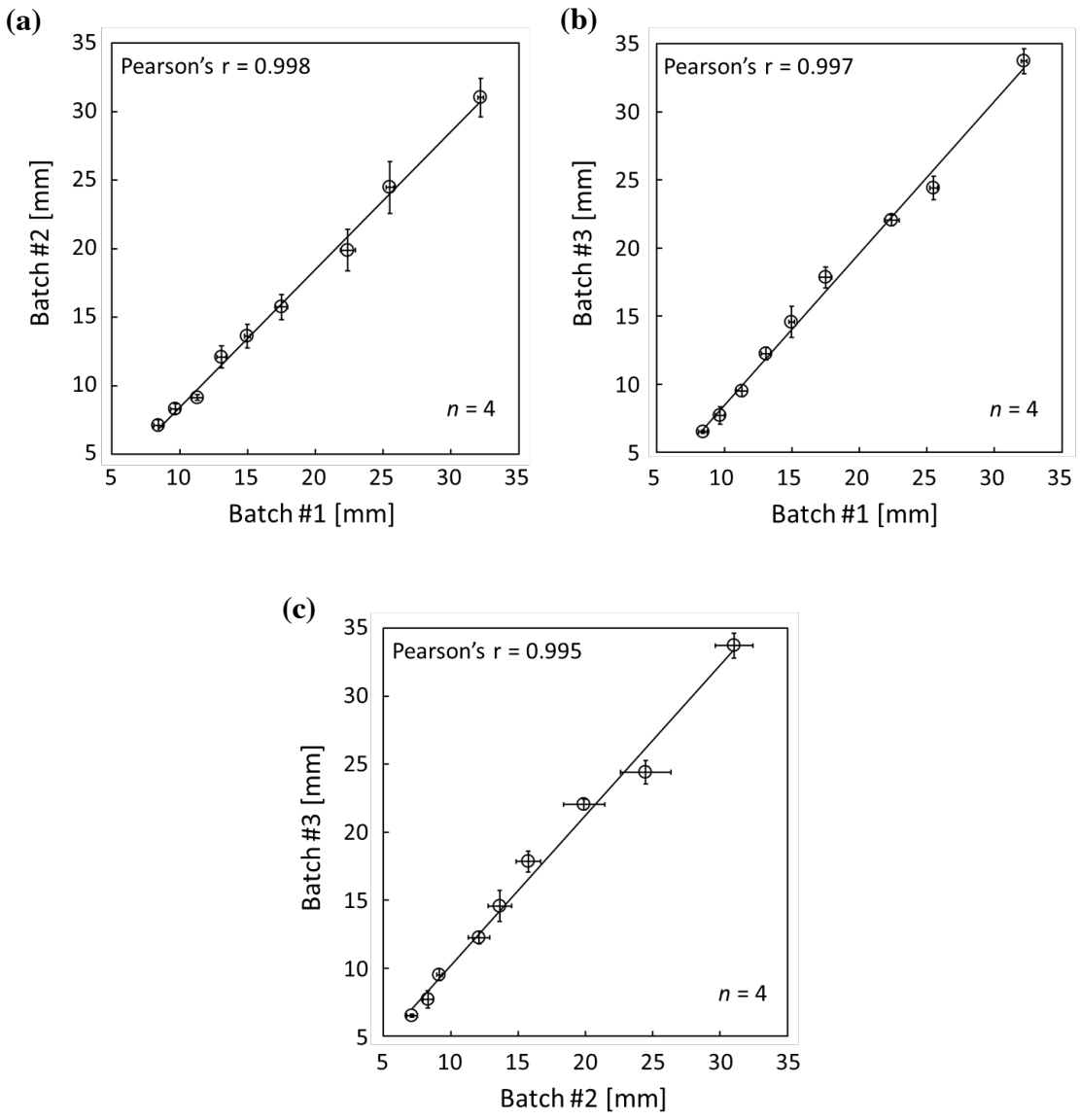


**Figure S9.** Comparison between different batches of fabricated µPADs; the underlying data is identical to Fig. 4a in the main text.

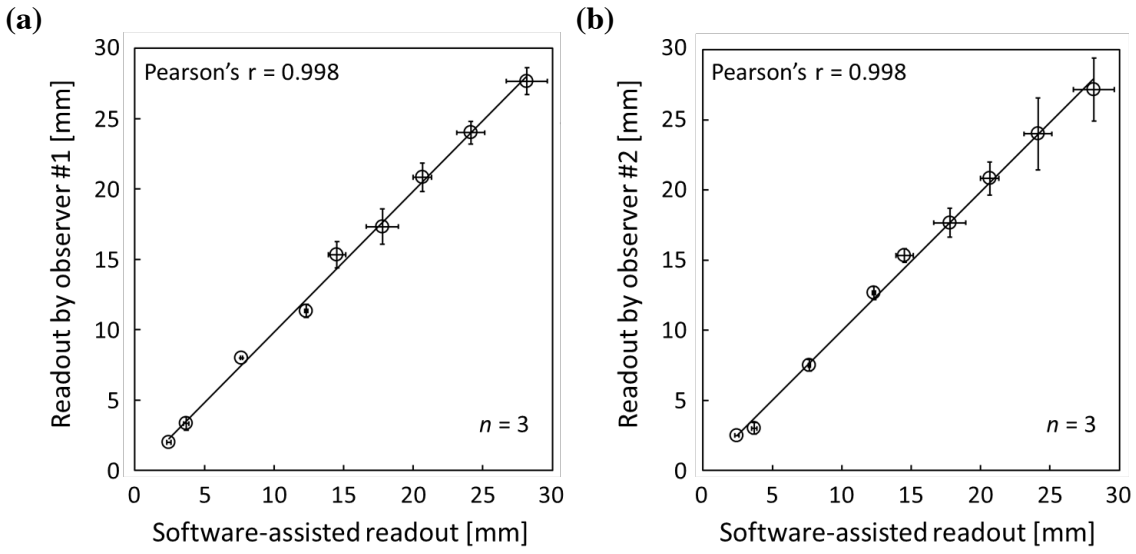


**Figure S10.** Comparison of $Ca^{2+}$ assay results with distance-based μPADs between software-assisted readout and readouts by two individual observers; the underlying data is identical to Fig. 4b in the main text.

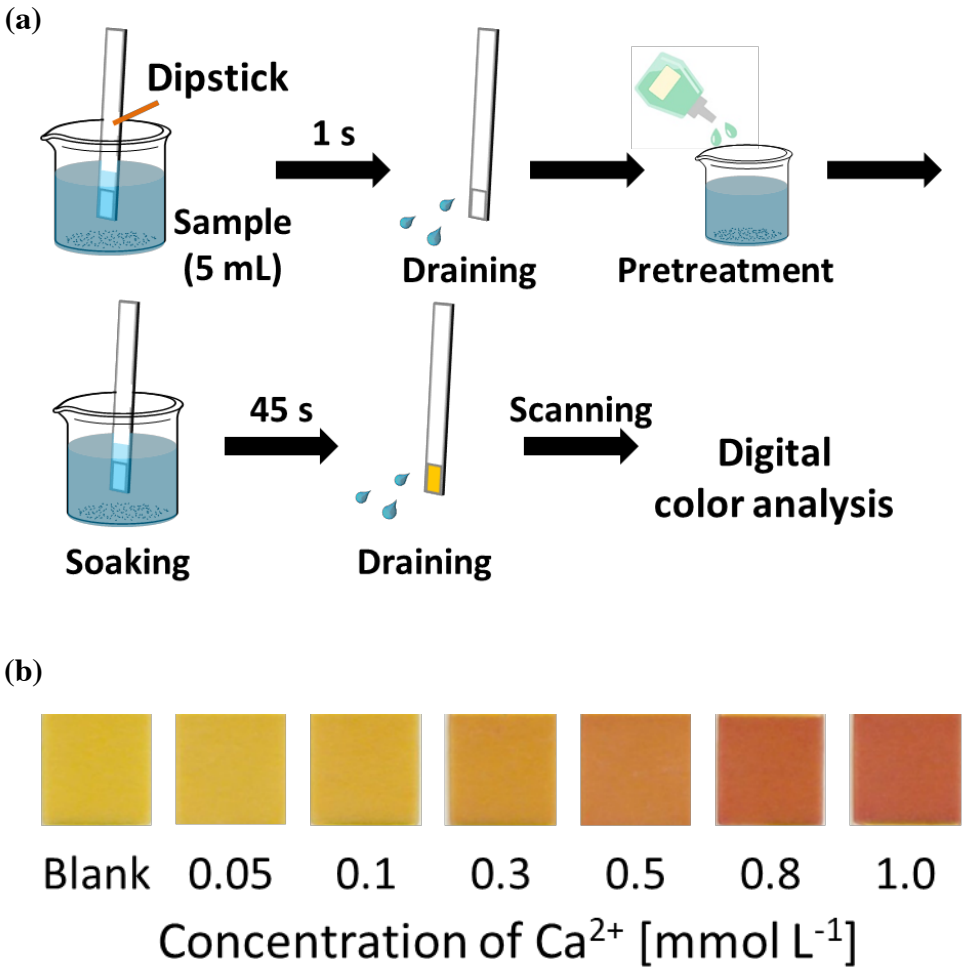


**Figure S11.** (a) Assay procedure applied for commercial colorimetric paper dipstick for $Ca^{2+}$ according to the attached user manual; (b) Scanned images of the commercial test strips after exposure to the corresponding $Ca^{2+}$ concentrations.

**Table S1.** Determination of the lowest naked-eye detectable $Ca^{2+}$ concentration with commercial paper dipsticks.

| Observer # | Concentration of $Ca^{2+}$ [mmol $L^{-1}$] | | | | |
|---|---|---|---|---|---|
| | 0 | 0.05 | 0.1 | 0.2 | 0.3 |
| #1 | - | - | ✓ | ✓ | ✓ |
| #2 | - | - | ✓ | ✓ | ✓ |
| #3 | - | - | ✓ | ✓ | ✓ |
| #4 | - | - | ✓ | ✓ | ✓ |

"-" and "✓" stand for "unobservable colour change" and "observable colour change", respectively, read out by 4 independent users; before comparing each sample exposed dipstick to a reference dipstick (*i.e.* exposed to blank), the colour code reference of the user manual was used for user instruction.

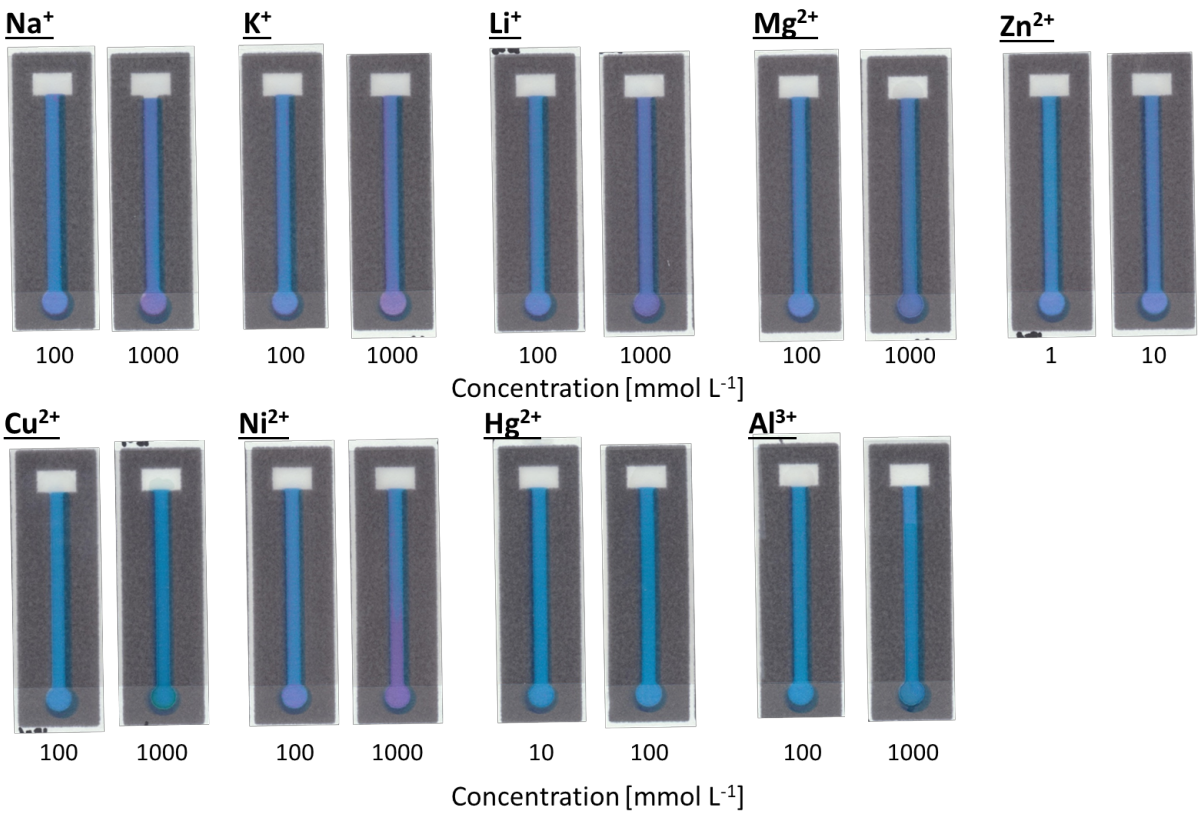


**Figure S12.** Scanned images of $Ca^{2+}$-selective μPADs exposed to the corresponding various cation concentrations for selectivity evaluation based on visual recognition; 30 μL of sample solutions prepared as chloride salts were applied onto μPADs; incubation time: 45 min.

**Competitive interference study using $Na^+$ and $Mg^{2+}$**

30 µL of aqueous $Ca^{2+}$ sample (1 mmol $L^{-1}$) containing various concentrations of $Na^+$ or $Mg^{2+}$ (0, 0.5, 1, 3 and 5 mmol $L^{-1}$) was applied onto the fabricated $Ca^{2+}$-selective µPAD. After the incubation for 45 min under ambient condition, the generated distance-based signal was quantified with ImageJ software in the same manner as for $Ca^{2+}$ assays demonstrated in section 2.4 of the main text.

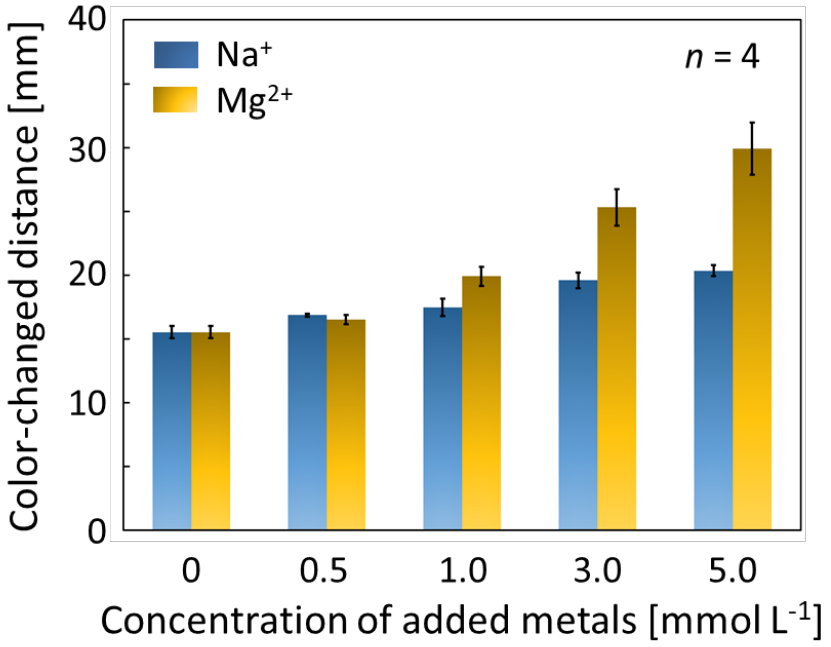


**Figure S13.** Result of $Ca^{2+}$ concentration readout in the presence of potentially interfering cations ($Na^+$ and $Mg^{2+}$). The amount of $Ca^{2+}$ was fixed at 1 mmol $L^{-1}$, whereas the amount of the interfering cations was varied (from 0 mmol $L^{-1}$ to 5 mmol $L^{-1}$); each data point has been obtained by measurements with 4 individual single-use distance-based µPADs; error bars indicate the standard deviations.

**Evaluation of the adsorption of $Ca^{2+}$ onto paper substrates**

30 µL of pH-buffered $Ca^{2+}$ samples (50 mmol $L^{-1}$ HEPES-TMAOH buffer, pH 7.0) was applied onto a reagent-free paper channel (device design is shown in Fig. S4a), followed by incubation for 45 min. Then, $Ca^{2+}$-selective nano-optodes were inkjet-printed onto the paper devices exposed to various concentrations of $Ca^{2+}$ samples at 20 printing cycles. After inkjet printing, colorimetric signals were captured with a colour scanner.

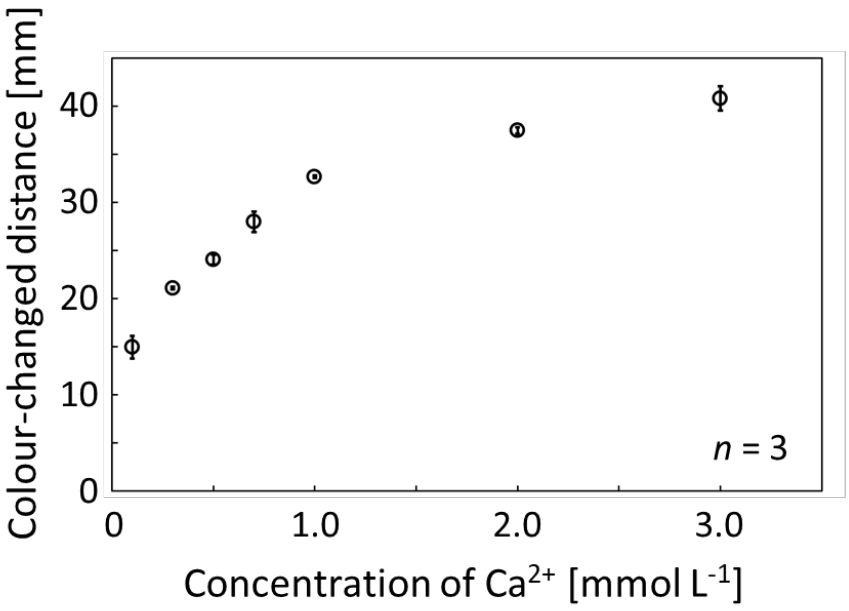


**Figure S14.** Software-assisted response curve to estimate the adsorption of $Ca^{2+}$ onto the paper substrate; each data point has been obtained by measurements with 3 individual single-use devices; 30 µL of aqueous $CaCl_2$ solution applied; error bars indicate the standard deviations; incubation time: 45 min.

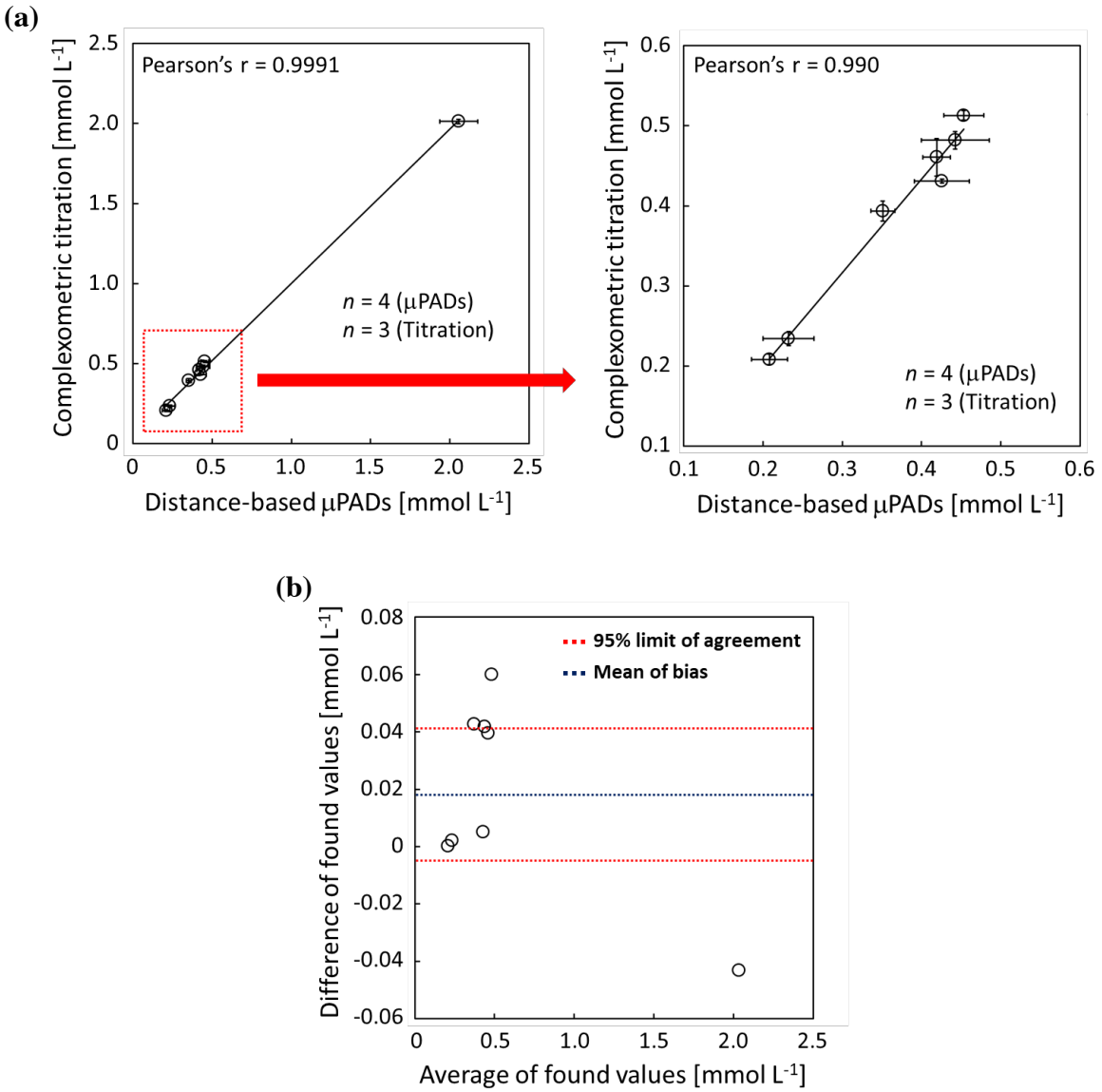


**Figure S15.** (a) Comparison between $Ca^{2+}$ measurements by distance-based μPADs and complexometric titration; the plots and error bars represent the average and standard deviations of 4 (μPADs) and 3 (titration) repetitions; (b) Bland-Altmann analysis for eight $Ca^{2+}$ samples quantified by μPADs and complexometric titration; the underlying data is identical to Table 2 in the main text.

**Table S2.** Errors in naked-eye quantification of $Ca^{2+}$ in unspiked or spiked tap water by untrained observers compared to ImageJ software-assisted readout results; different batches of μPADs were used for data shown in parts (a) and (b) of the table; the data represent the mean and standard deviations of 3 independent readouts by a total of 24 observers.

**(a)**

| Sample | Unspiked [%] | Spiked #1 [%] | Spiked #2 [%] | Spiked #3 [%] |
|---|---|---|---|---|
| Observer #1 | +8±3 | 0±1 | 0±0 | -6±0 |
| Observer #2 | +1±0 | +5±2 | +5±4 | -1±0 |
| Observer #3 | +8±5 | -1±0 | +2±3 | -4±3 |
| Observer #4 | +12±0 | +1±4 | +2±3 | -1±0 |
| Observer #5 | -3±5 | -1±0 | +2±3 | -2±3 |
| Observer #6 | +12±0 | +9±7 | +2±3 | -2±3 |
| Observer #7 | -3±5 | +4±4 | +2±3 | -3±3 |
| Observer #8 | +3±3 | -4±2 | +2±1 | -3±1 |
| Observer #9 | -1±5 | -5±3 | -1±3 | -3±0 |
| Observer #10 | -1±3 | -1±0 | +3±3 | -1±0 |
| Observer #11 | +12±5 | +5±2 | +2±6 | +1±3 |
| Observer #12 | +10±3 | +9±2 | -4±11 | +2±2 |
| Observer #13 | +4±3 | +3±3 | -1±3 | 0±1 |
| Observer #14 | +23±5 | +14±0 | +6±0 | +1±3 |

**(b)**

| Sample | Unspiked [%] | Spiked #1 [%] | Spiked #2 [%] | Spiked #3 [%] |
|---|---|---|---|---|
| Observer #15 | +12±0 | +12±2 | +11±5 | +3±0 |
| Observer #16 | +2±4 | +2±6 | -2±4 | 0±3 |
| Observer #17 | -11±5 | +1±5 | -2±2 | -3±3 |
| Observer #18 | +6±6 | +3±6 | -3±0 | 0±3 |
| Observer #19 | -11±2 | -3±5 | -5±2 | -4±3 |
| Observer #20 | -8±4 | -2±3 | +2±2 | -3±5 |
| Observer #21 | +1±2 | +6±5 | 0±1 | -6±2 |
| Observer #22 | -6±2 | -1±5 | +1±2 | -5±2 |
| Observer #23 | +2±8 | +4±8 | +4±0 | 0±1 |
| Observer #24 | -1±5 | +1±5 | 0±0 | -2±2 |