## Quantitative particle agglutination assay for point-of-care testing using mobile holographic imaging and deep learning

## **Supplementary Information**

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## **Supplementary Figures**



\* Total assay cost: 20.8¢/test (including test bead and CPR antigen cost)

**Fig. S1**. The dual-channel capillary lateral flow device. (a) Top and bottom design. (b) Device image. (c) Images of the optimized materials and device holder. (d) The total cost of used materials for the assembly of a single test (unit:  $\phi$ ). The total assay cost per test is 20.8  $\phi$  with the inclusion of CRP antigen and test particles' cost.



**Fig. S2.** Neural network structure and training log for (a) the classification neural network and (b) the quantification neural network. The gray dashed vertical lines in both plots indicate the selected network model based on the minimum validation loss.



**Fig. S3**. Comparison of the control channel agglutination activity according to the control particle preparation method (antigen saturated, heat-treated, and UV-exposed). Microscope images of the test and control channels for low CRP concentration samples with high false-positive agglutination (a), and high-CRP concentration ( $5.4\mu g/mL$ ) without false-positive agglutination (b). In the case of antigen-saturated test beads, false-positive reactions could be distinguished regardless of the CRP concentration. Heat-treated and UV-exposed particles were not distinguished from false-positive agglutination. In the case of BSA-coated particles, agglutination reaction was not observed in samples in which non-specific agglutination reaction was observed (data not shown). The heat-treated particles were prepared by incubation in a boiled water chamber for 30 min, and the UV-exposed particles were prepared by exposure under the UV lamp ( $6.5mW/cm^2$ ) for 1 hour.



**Fig. S4** (a) Comparison of the flow rate at the capillary channels as a function of the external humidity (n=3). The flow rates were obtained within a humidity-controlled digital balance by measuring the weight change over time. (b) Comparison of CRP concentration measurement results as a function of the flow rate (n=3 at 25% external humidity). CRP concentrations were measured for various sizes of adsorption membrane through the quantification neural network inference, and % Bias (y-axis) represents the difference from the CRP concentration value that is measured using the optimized membrane size (red mark: 16mm<sup>2</sup> of adsorption membrane).



**Fig. S5**. Time evolution of the total particle area inside the test channels for low (1.1  $\mu$ g/mL) and very high (200  $\mu$ g/mL) CRP concentration samples.



**Fig. S6** Time evolution of the total particle area (a) and the total particle number (b) (normalized by channel width) inside both the test (first row) and the control channels (second row). Measurements on different CRP concentration levels (insert box unit:  $\mu g/mL$ ) were displayed for example. The green curves correspond to one of the serum samples that was represented by the green dots in Fig. 4 of main text.