Rapid and highly sensitive immunoassay using an ultra-thin immuno-wall microfluidic device with a sequential fluorescence signal increment method

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Supplementary Material I

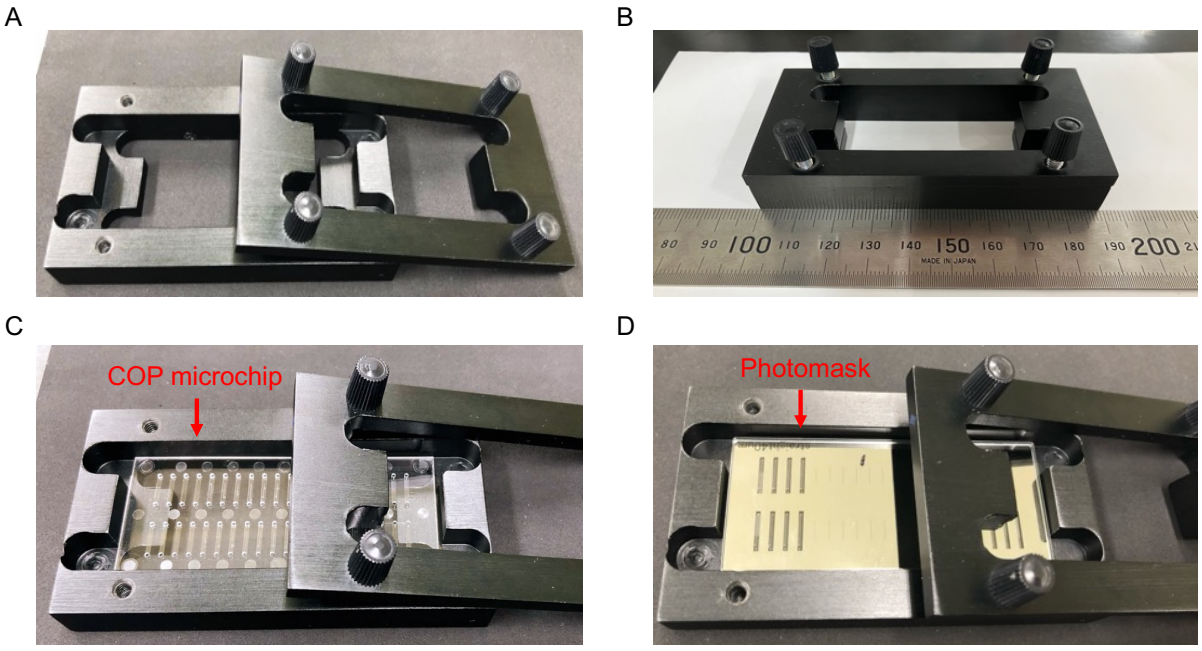


Fig. S1 (A) Photograph of alignment holder. (B) Alignment holder with a scale (smallest division: 1 mm). (C) COP chip inside alignment holder. (D) COP chip with aligned photomask inside alignment holder.

Supplementary Material II

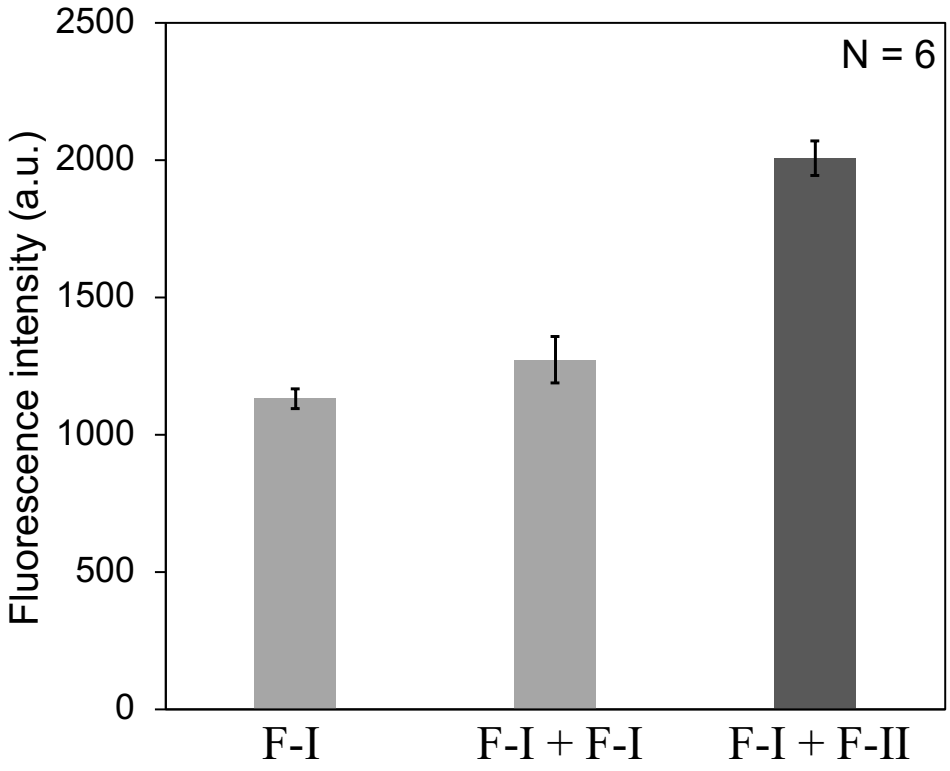


Fig. S2 Bar graph of the confirmation of the formation of multi-layer immune complex.

(S-protein: 100 ng/mL)

Supplementary Material III

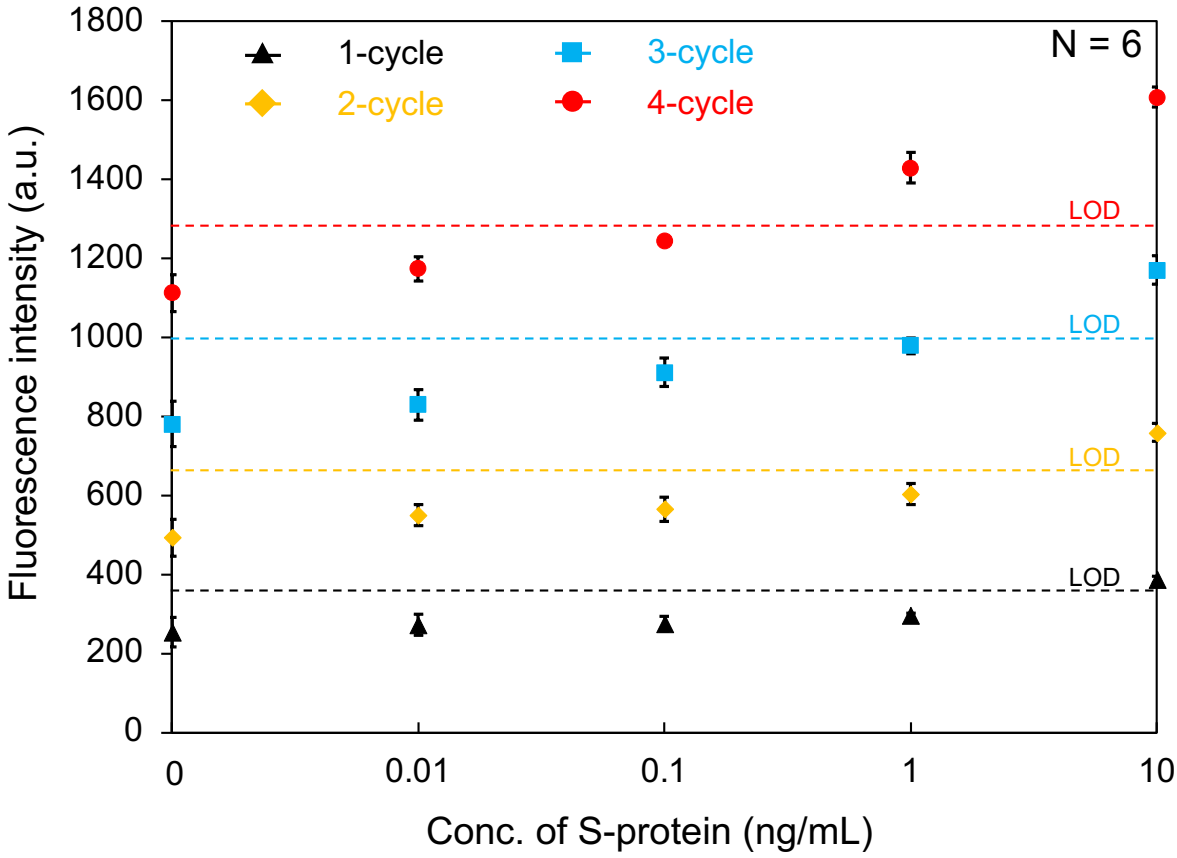


Fig. S3 Plot of fluorescence intensity vs concentration of S-protein in 0.5% BSA/PBS buffer with different incubation cycles of fluorescence-labeled antibody using conventional immuno-wall. (1-cycle: F-I → 2-cycle: F-II → 3-cycle : F-I → 4-cycle : F-II). The value of fluorescence intensity was plotted as the mean ± 3SD (N = 6). The dotted line represents the LOD which is 3 times of SDs above the negative control (0 ng/mL).

Supplementary Material IV

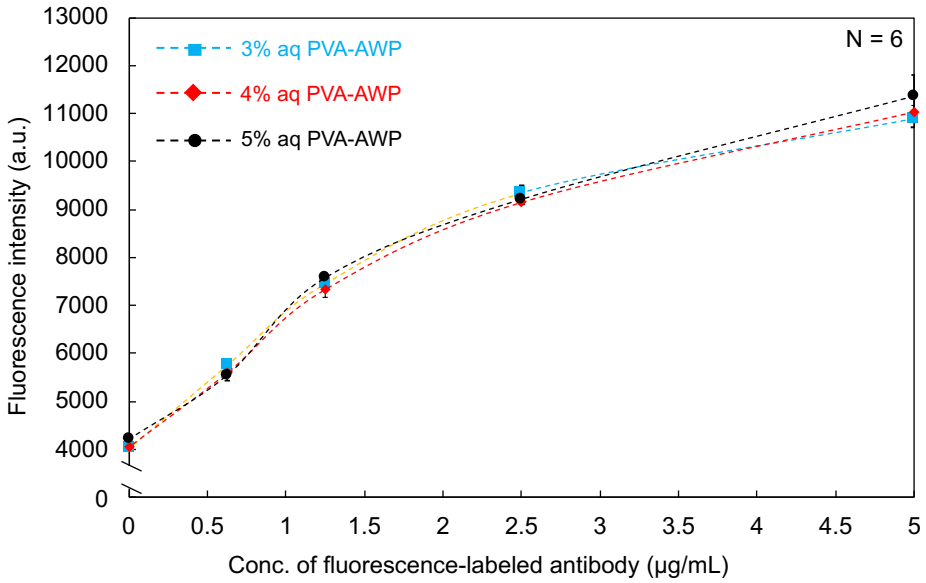
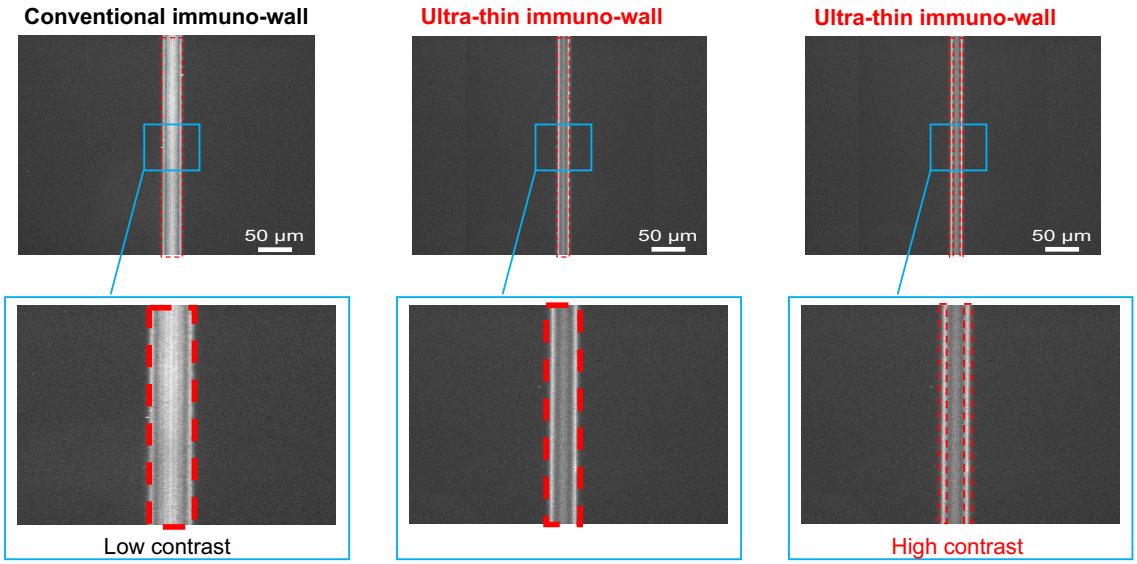


Fig. S4 Plot of fluorescence intensity vs concentration of fluorescence-labeled antibody (5, 2.5, 1.25, 0.625 μg/mL) with different concentrations of PVA-AWP. In this experiment, 50 μg/mL immobilized antibody was used for immobilization. To estimate the immobilization ability of the immuno-wall with different cross-linking densities, DyLight 650-conjugated rabbit anti-human polyclonal IgG antibody diluted as 5, 2.5, 1.25, and 0.625 μg/mL was used to react with immobilized antibodies.

Supplementary Material V



$$Fluorescence\ intensity\ of\ each\ image = \frac{\sum Value\ of\ each\ pixel\ in\ selected\ area}{number\ of\ pixel}$$

❖ *The mean value of the fluorescence intensity increases when the lateral-side area is selected as the detection area, due to the decreased number of pixel from the middle area of immuno-wall.*

Fig. S5 Fluorescence intensity analysis based on different detection areas.