Electronic Supplementary Material (ESI) for Lab on a Chip.

Lab on a Chip

Supplementary Information

Title: One-step immunoassay of C-reactive protein using droplet microfluidics

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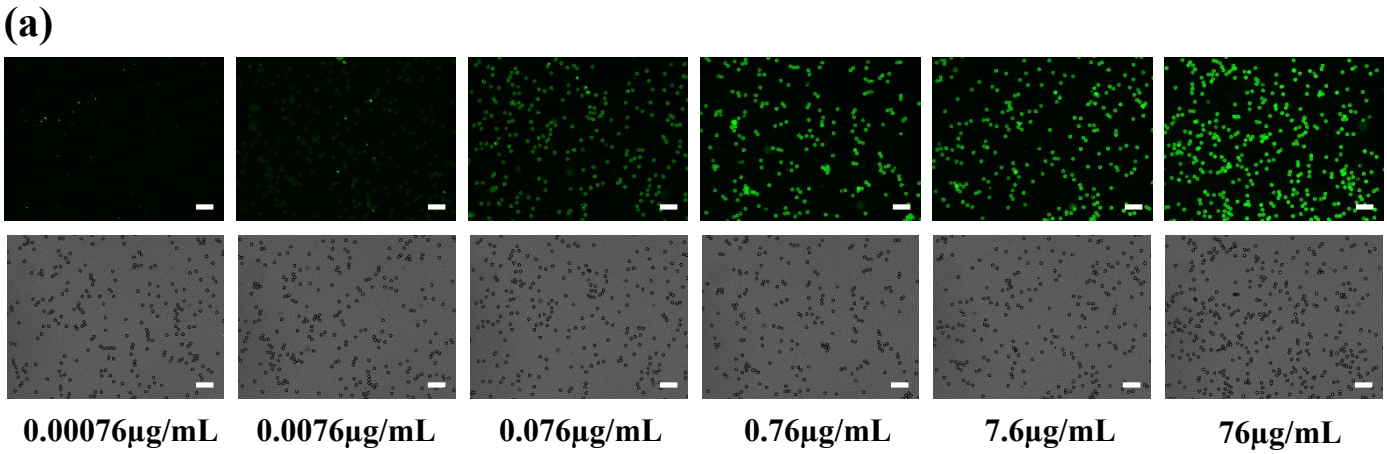
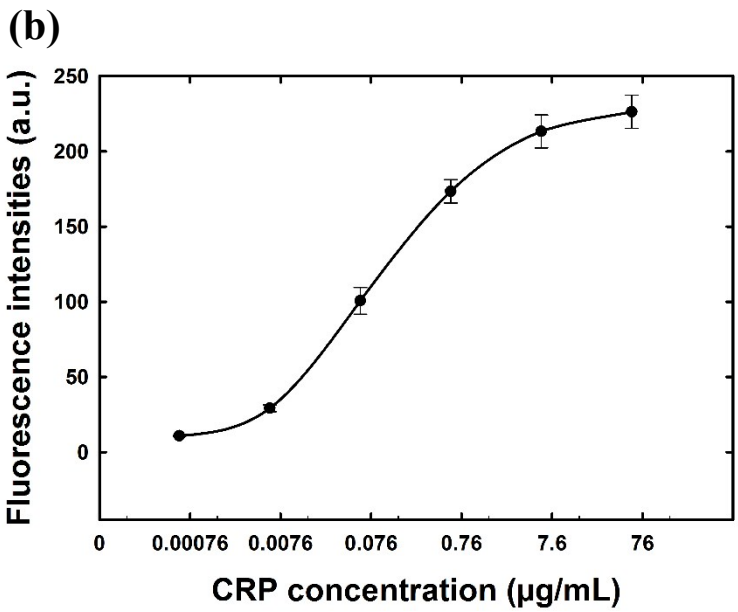
S.1 Characterization of the immunoassay by the bead fluorescence.

The fluorescence intensities of the microbeads become higher as the CRP concentration increases, suggesting that the sandwiching effect remains after the capture antibodies are coupled onto the microbeads. (a) Fluorescence images and bright-field images of the microbeads at different CRP concentrations of 0.00076, 0.0076, 0.076, 0.76, 7.6, and 76μg/mL. Scale bar: 50μm. (b) A plot of the fluorescence intensities as a function of CRP concentration. More than 30 beads were analyzed for each CRP concentration.

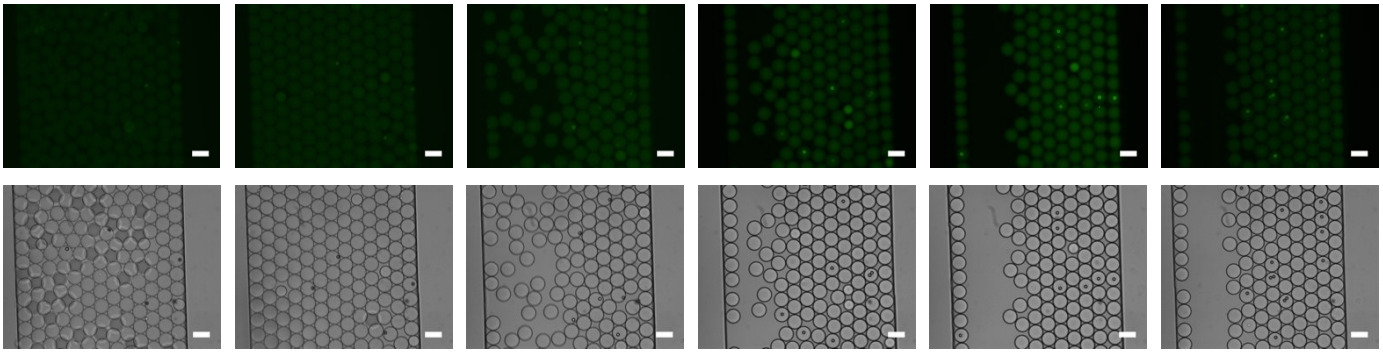




S.2 Measurement of the incubation time for the immunoassay within droplets.

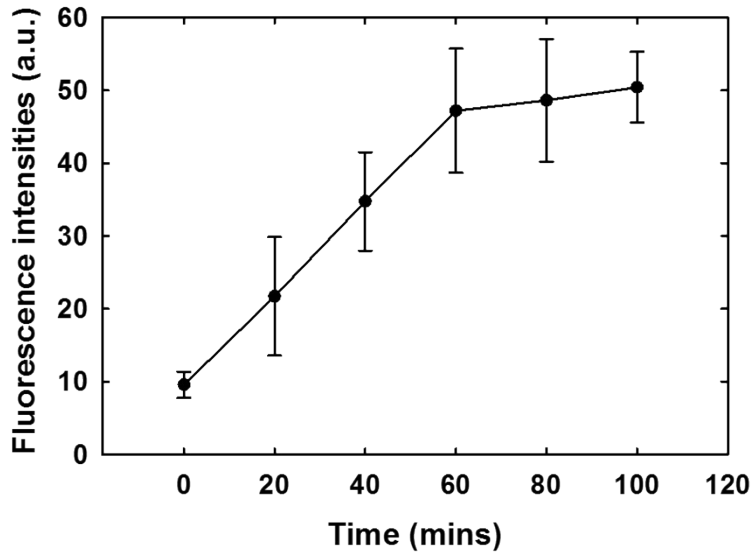
The microbeads, CRP, and detection antibodies are confined within a droplet and washing steps are no longer introduced easily. Therefore, the interaction between the antibodies can hinder the antibody-antigen binding and it will take a longer time for the sandwiching effect to take place. (a) Fluorescence images and bright-field images of the droplets at different incubation time points of 0, 20, 40, 60, 80, 100 minutes. Scale bar: 50μm. (b) A plot of the bead fluorescence as a function of incubation time. More than 30 droplets with beads were analyzed.

(a)



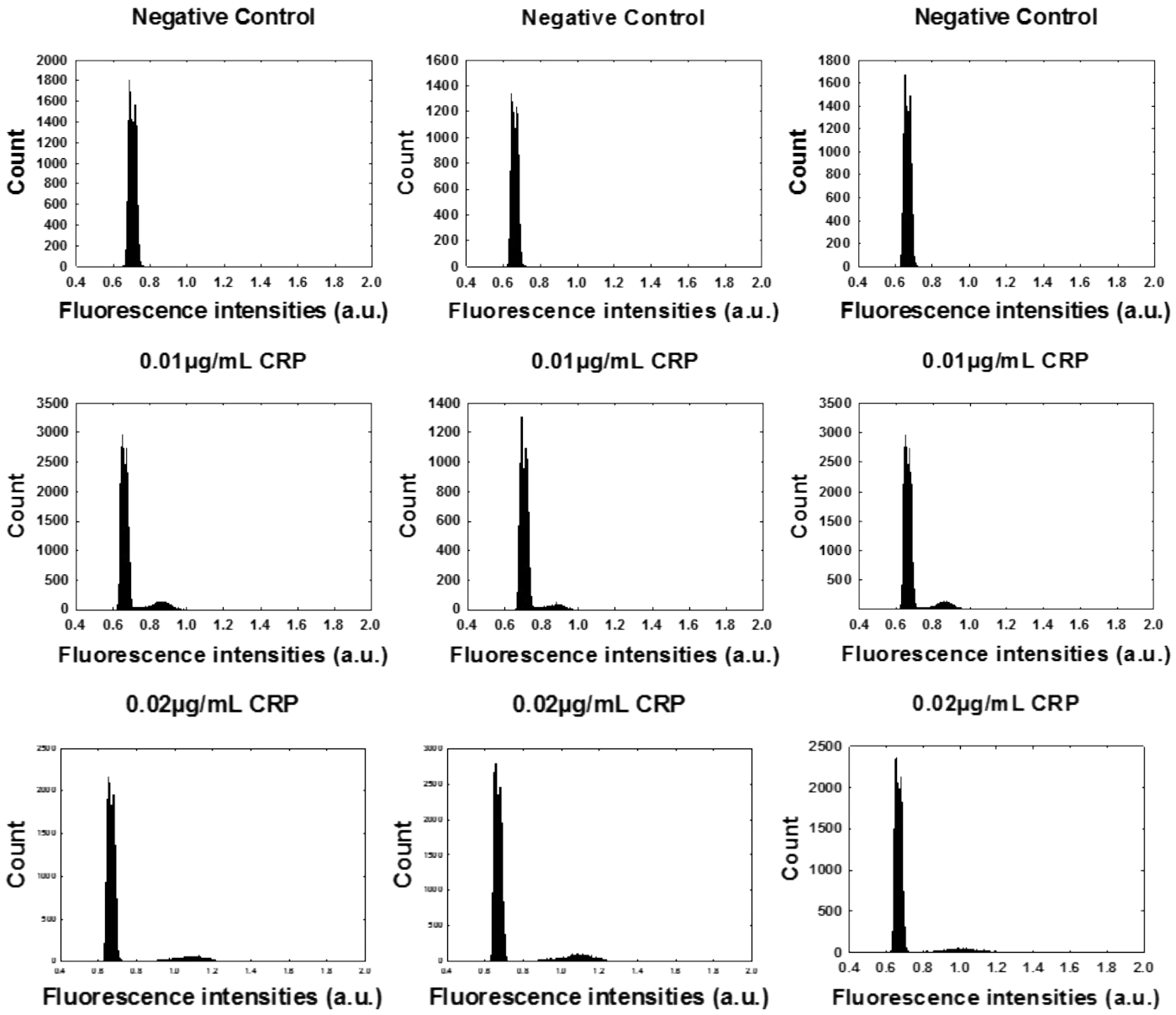
0 mins 20 mins 40 mins 60 mins 80 mins 100 mins

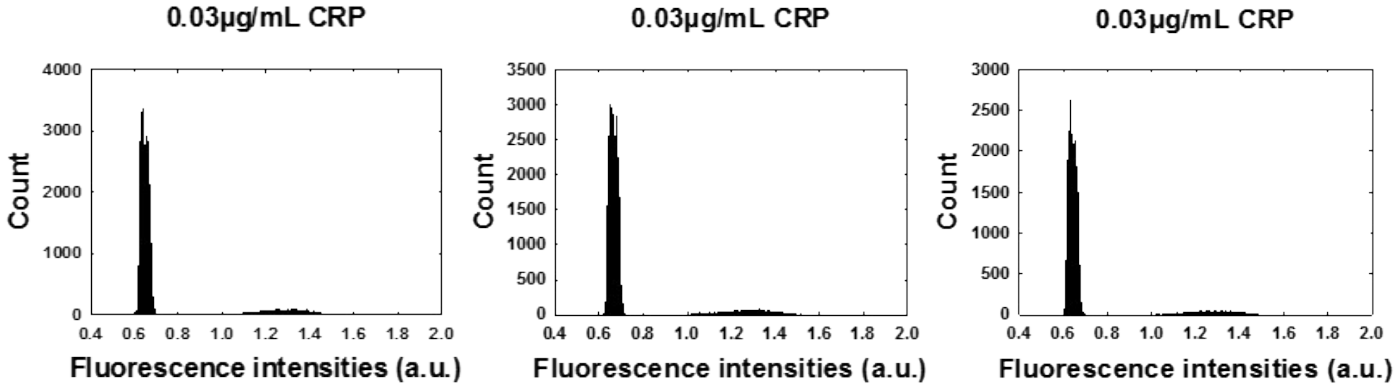
(b)

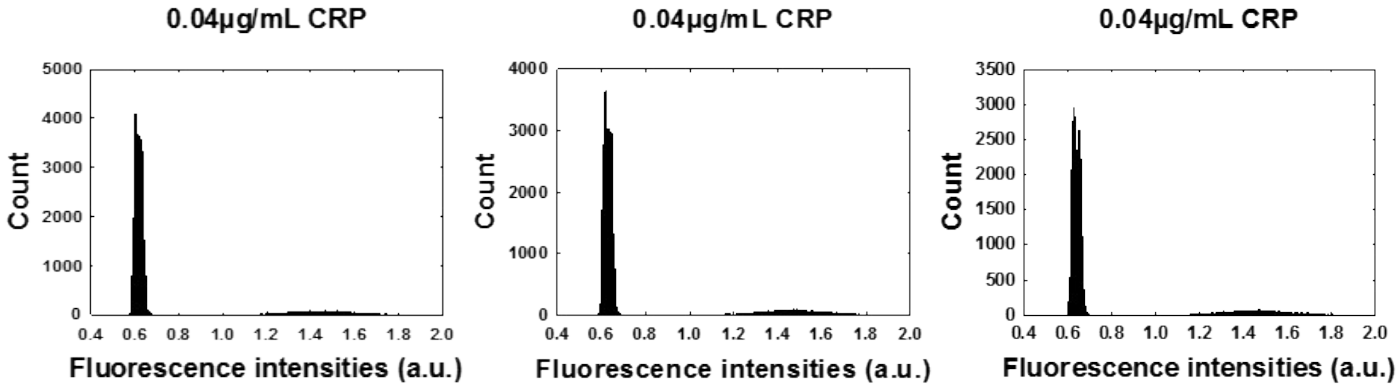


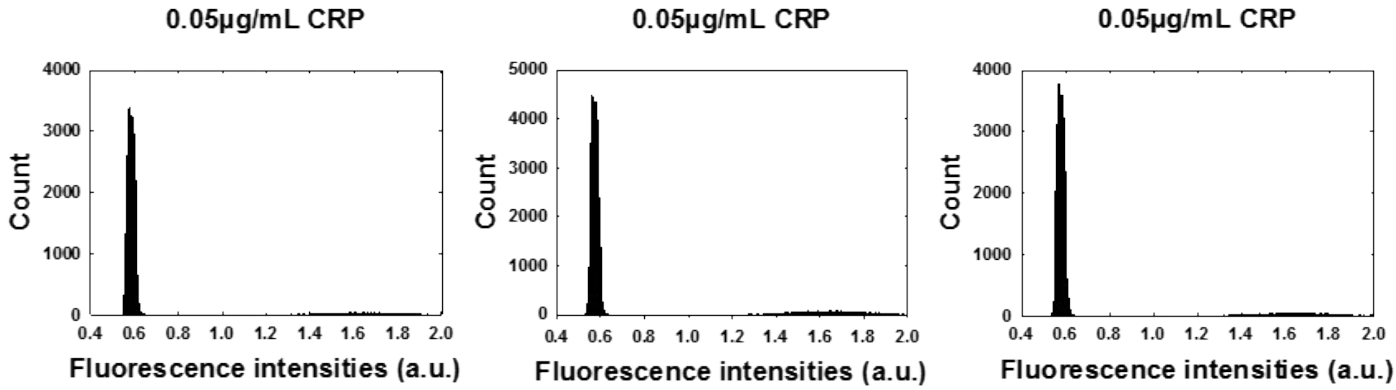
S.3 Quantitative analysis of droplet intensities by the detection platform.

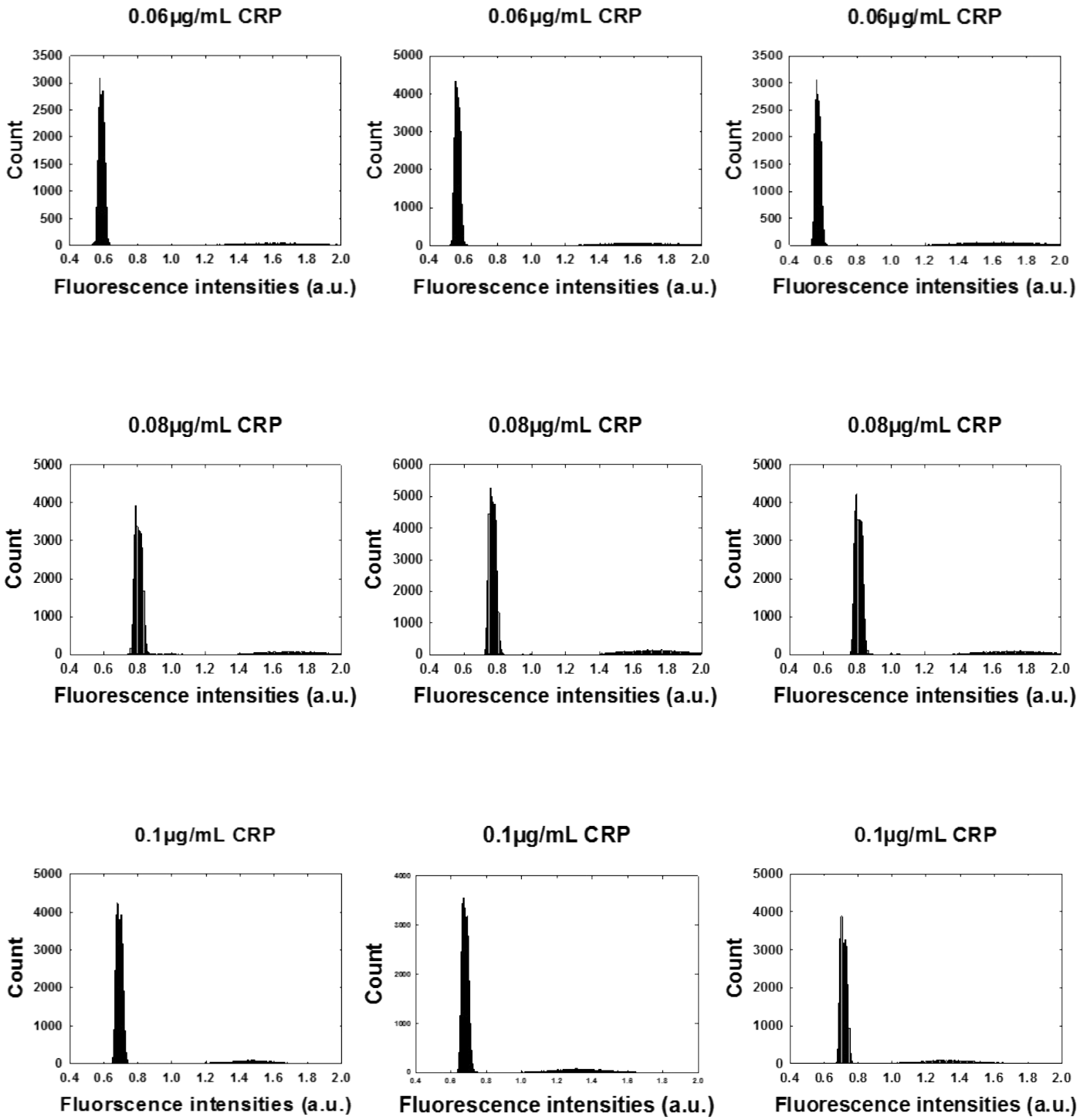
S3.1 Laser line scanning of the droplet fluorescence by the detection platform. The negative control shows the background fluorescence from the fluorophores of the detection antibodies, hence defining the lowest fluorescence intensities our droplet system can provide. The peak fluorescence of the droplets is measured by selecting the highest value of the fluorescence within a user-defined time width. The time width is defined by the duration a droplet travels under the laser line, as confirmed by the high-speed imaging performed. The three columns of data set below indicate three different experimental runs, with different CRP concentration of 0 (negative control), 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, and 0.1μg/mL. The fluorescence intensities of the high-intensity peaks are plotted below, with the value of the intensities listed in table S3.1. A statistical t-test is performed to statistically quantify the ability of our detection system in differentiating different CRP concentrations, as listed in table S3.2. The number of droplets analyzed per experimental run per concentration is more than 500.




0.03μg/mL CRP
0.03μg/mL CRP
0.03μg/mL CRP
Count
Fluorescence intensities (a.u.)


0.04μg/mL CRP
0.04μg/mL CRP
0.04μg/mL CRP
Count
Fluorescence intensities (a.u.)


0.05μg/mL CRP
0.05μg/mL CRP
0.05μg/mL CRP
Count
Fluorescence intensities (a.u.)


0.06μg/mL CRP
0.06μg/mL CRP
0.06μg/mL CRP
0.08μg/mL CRP
0.08μg/mL CRP
0.08μg/mL CRP
0.1μg/mL CRP
0.1μg/mL CRP
0.1μg/mL CRP
Count
Fluorescence intensities (a.u.)
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Fluorescence intensities (a.u.)

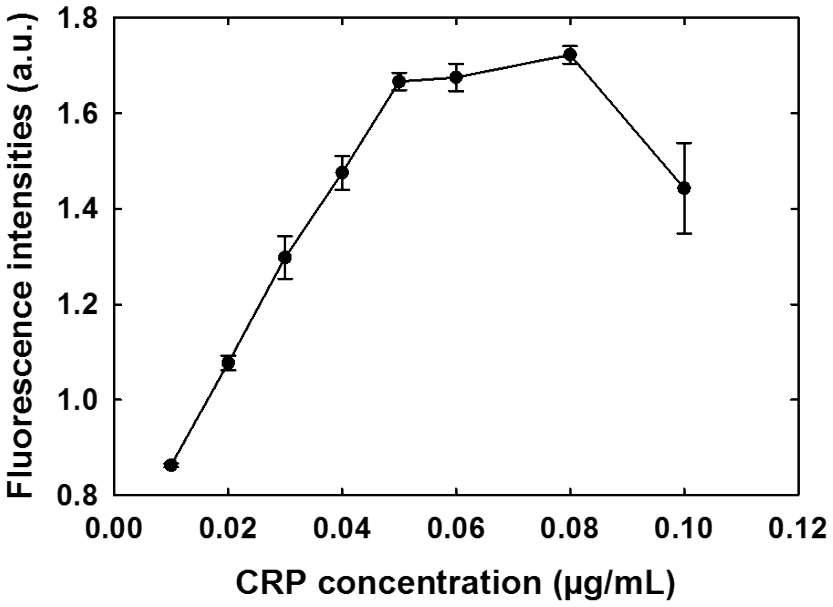
Table S3.1. Fluorescence intensities of the high-intensity peaks of the three experimental runs.

CRP concentrations (μg/mL)	Mean fluorescence intensities (a.u.)			Standard deviation		
	1st run	2nd run	3rd run	1st run	2nd run	3rd run
0.01	0.859755	0.862076	0.86673	0.04936	0.0548	0.054395
0.02	1.063879	1.0943	1.072785	0.139966	0.134796	0.130662
0.03	1.358068	1.263716	1.282172	0.232111	0.134737	0.127406
0.04	1.499477	1.491497	1.434878	0.209096	0.186795	0.168256
0.05	1.671983	1.680524	1.646093	0.224174	0.22901	0.202973
0.06	1.681163	1.691768	1.643468	0.271039	0.249908	0.26151
0.08	1.700917	1.734605	1.730606	0.180132	0.181159	0.192477
0.1	1.541372	1.352444	1.434662	0.317166	0.190473	0.324566

Table S3.2. To quantify how well our detection system can differentiate between CRP concentrations, a statistical analysis with independent two samples t test is used. The null hypothesis is defined as when there is no difference between the mean values of two CRP concentrations, with a 95% confidence interval. The p values of the statistical test are listed as follow:

CRP concentration comparison	P Value			Statistical Significant?		
	1st run	2nd run	3rd run	1st run	2nd run	3rd run
0.01μg/mL and 0.02μg/mL	<0.0001	<0.0001	<0.0001	Yes	Yes	Yes
0.02μg/mL and 0.03μg/mL	<0.0001	<0.0001	<0.0001	Yes	Yes	Yes
0.03μg/mL and 0.04μg/mL	<0.0001	<0.0001	<0.0001	Yes	Yes	Yes
0.04μg/mL and 0.05μg/mL	<0.0001	<0.0001	<0.0001	Yes	Yes	Yes
0.05μg/mL and 0.06μg/mL	0.1745	0.0663	0.7588	No	No	No
0.06μg/mL and 0.08μg/mL	0.0006	<0.0001	<0.0001	Yes	Yes	Yes
0.08μg/mL and 0.1μg/mL	<0.0001	<0.0001	<0.0001	Yes	Yes	Yes

S3.2 A plot of the droplet fluorescence intensities as a function of CRP concentration. The data in the plot represents the average of three experimental runs, with the population of droplets with the high fluorescence intensity in figure S3.1 analyzed. Here, the fluorescence intensities of the droplets are scanned by the laser line of our platform after incubation for 60 mins. Values shown below indicate the fluorescence intensities concentrated on the microbead within the droplets. The fluorescence intensities plateau when the CRP concentration is at 0.06-0.08μg/mL, and decrease when the concentration of CRP increases further. The excess CRP competes with the captured antibodies for the limited detection antibodies within droplets, thus preventing the antibodies from binding onto the microbeads to form the sandwiching complexes. The phenomenon, known as the prozone effect, is prevalent in one-step immunoassays when all the antibodies and excess analytes are present[1].



Reference:

1. J. Tate and G. Ward, *Clin Biochem Rev*, 2004, **25**, 105-120.