Supporting Information for

Large-scale femtoliter droplet array for digital counting of single-biomolecules

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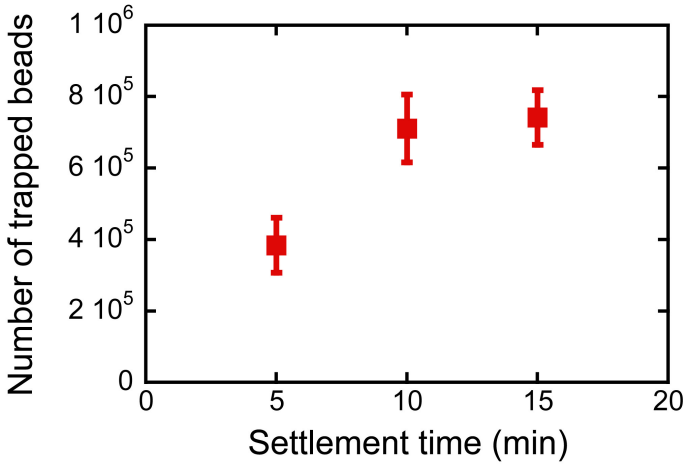
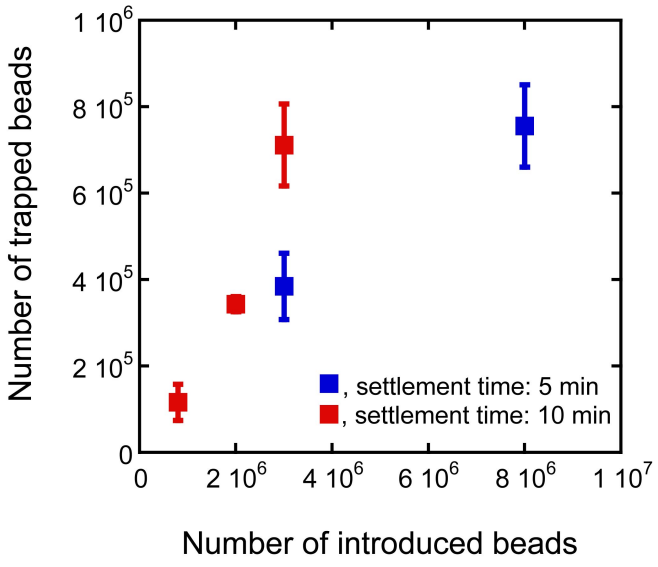
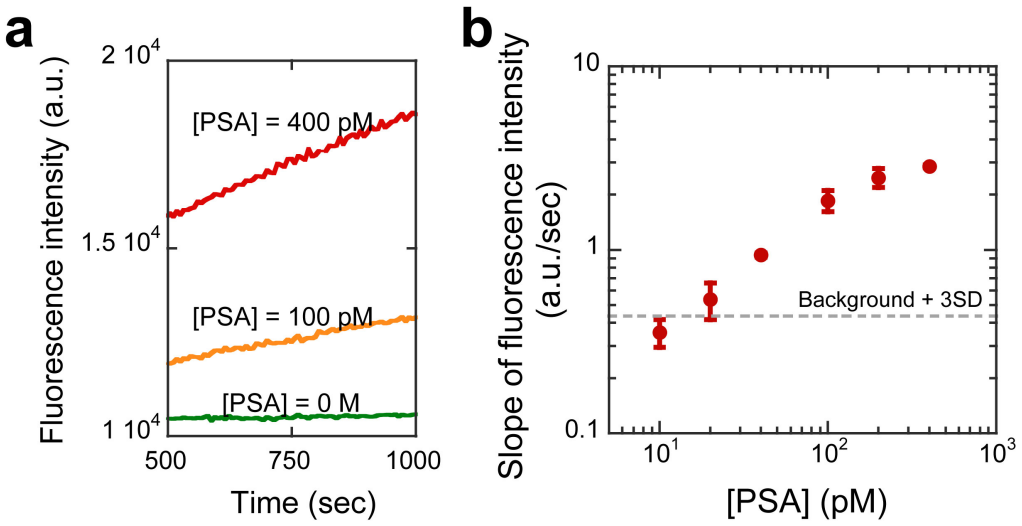


Supplementary Figure S1. Beads trapping efficiency depending on the settlement time. For the investigation of beads trapping efficiency depending on the settlement time, 3×10^6 beads were introduced into the device. The beads were settled for several minutes after removing gases from the microwell. The number of trapped beads reached a plateau around 10 min of settlement time.



Supplementary Figure S2. Beads trapping efficiency depending on the number of introduced beads. For the investigation of beads trapping efficiency depending on the number of introduced beads, various numbers of beads were introduced into the device. The number of trapped beads increased with the number of introduced beads with 10 min of settlement time. However, if we introduce large number of beads (8×10^6) with 10 min of settlement time, two or three beads were often trapped into a single droplet, while 5 min of settlement time decreased fraction of droplet containing multiple beads. These results illustrate how we can efficiently trap beads with different number of introduced beads by controlling settlement time.



Supplementary Figure S3. Results of bead-based conventional ELISA. A fluorescence plate reader was used for the detection of the signal from ensemble of the beads, where the slope of the fluorescence signal was measured for each [PSA]. LOD was determined to be 14 pM.

Supplementary Table 1. Comparison of the results for digital ELISA of PSA

Total number of reactors	LOD	Background (%)	SD (%)	Measurement CV	Average number of reactors for background	Poisson noise CV
1,000,000 Present study	2 aM	0.3	0.01	3%	2183	2%
100,000 Present study*	13 aM	0.3	0.04	12%	225	7%
8,600 Present study*	37 aM	0.3	0.13	38%	22	21%
50,000 Rissin *et al.*[14]	50 aM	0.5	0.19	36%	~135	~9%
200,000 Kan *et al.*[15]	370 aM	~0.7	0.06	8%	~175	~8%
		~0.7	0.12	18%		

* Randomly selected 12 or 1 blocks of array was used for the analysis of background.

Supplementary Video S1. Demonstration of sample injection, droplet formation, and bead enclosure. The video shows the demonstration of beads and oil injection using conventional micropipette. For the demonstration, highly concentrated polystyrene beads were suspended in aqueous solution and injected into the access port with a conventional micropipette. The device was first filled with the aqueous solution, then fluorinated oil was injected into the access port. The beads with aqueous solution were swept out by injecting oil into the device, concomitant with the droplet formation and bead enclosure.

Supplementary Video S2. Example of digital counting. The video shows representative fluorescence and bright-field images of each block. Biotinylated beads (3×10^6 particles) were mixed with 100 aM SβG in a 1 mL test tube. The beads were washed and trapped into the droplet array with FDG. After incubation, we acquired the fluorescence and bright-field images of each bock by scanning the device.