Electronic Supplementary Material (ESI) for Analyst.

SUPPORTING INFORMATION

Single-Molecule ELISA Device utilizing Nanofluidics

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S1 ELISA system and protocol

The system is shown in Figure S1. Control of flow velocity is essential in ELISA to control the reaction time. Consequently, pressures applied to the sample/reagent vessel were controlled to achieve the desired flow velocity at the micro/ nano channels. The sampling volume was controlled by regulating the time duration and an air pressure controller was used for fluidic control. An air-pressure fluidic control system was used to provide fluidic control at fL/s and the sample volume was controlled by the flow rate and duration of the application time. Single molecule sensitivity was achieved by using an enzymatic reaction time of 60 s. This generated 3.6 μM colored TMB after confinement, which could be detected by the DIC-TLM. The details of the design achieving single-molecule detection are shown in section 3 and include a histogram of the peak width after diffusion.

The operation and pressure sequence are shown in Table SI.

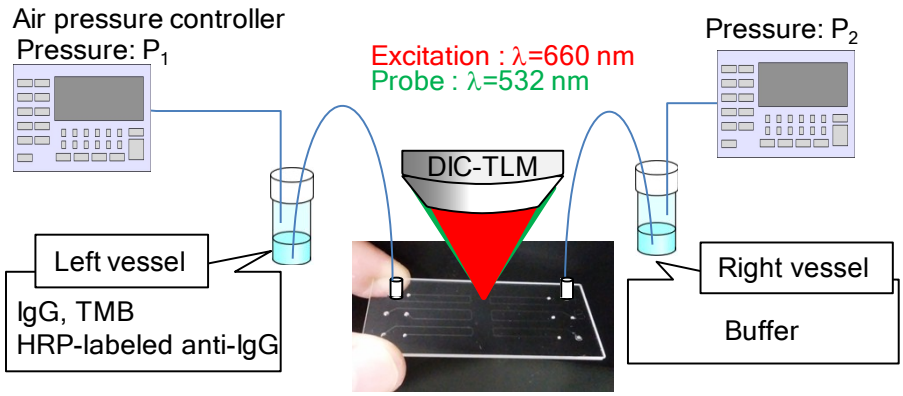

Figure S1. Experimental setup for nano-ELISA

Table SI Pressure sequence for nanofluidic ELISA

Operation	Left vessel		Right vessel		Time duration (s)
	Sample/Reagent	Pressure P_1 (kPa)	Reagent	Pressure P_2 (kPa)	
Introduction to microchannel	Analyte solution	450	Washing buffer	500	240
Sampling & immunoreaction		30 (average velocity 70 μm/s)		0	90
B/F separation		0		250	30
Introduction to microchannel	HRP-antibody solution	450		500	240
Introduction to nanochannel & immunoreaction		30 (average velocity 70 μm/s)		0	90
B/F separation		0		250	30
Introduction to microchannel	TMB solution	450		500	240
Introduction to nanochannel		500		0	60
Enzymatic reaction		0		0	60
Washing		250		0	60

S2 Calibration of average flow velocity and DIC-TLM sensitivity

Quantitative measurement requires calibration to guarantee the accuracy of the measurement. Several factors affect the accuracy of the nanofluidic ELISA system, namely, the flow velocity and sensitivity of the DIC-TLM. Flow velocity was calibrated by measurements before the assay. Colored TMB (20 μM) was introduced into the nanochannels and the times t_1 and t_2 for the signal rise were measured at two points (100 μm from the inlet and 100 μm from the outlet; distance d between the two points: 5800 μm). The average flow velocity $\bar{v}$ was calculated from $d/(t_1 - t_2)$. The applied pressure was adjusted to realize the desired average flow velocity $\bar{v}$. The sensitivity of the DIC-TLM is altered by changing the optical alignment between the nanochannel and the beam spot. The position and sensitivity were adjusted by introducing colored-TMB (20 μM) and the beam position was correctly aligned by measuring the signal intensity.

S3 Signals for single molecule ELISA

All the signals in Figure 4a are shown in Figure S2. The experimental conditions are same.

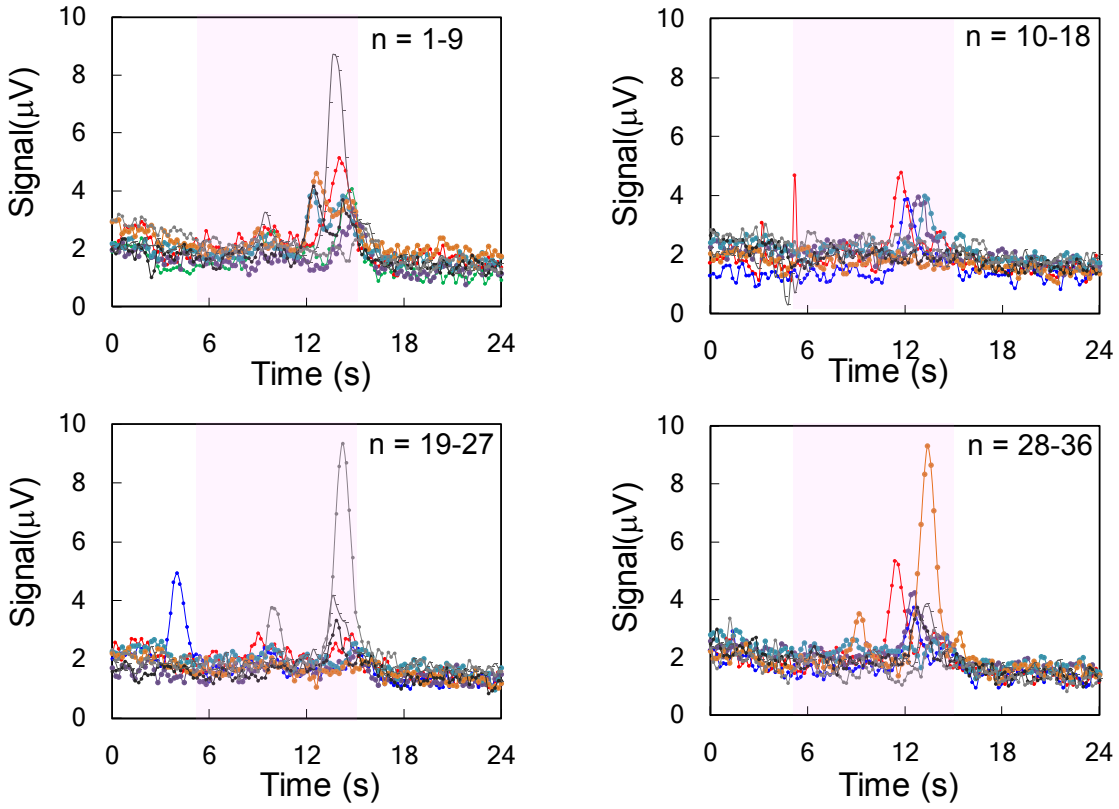

Figure S2. Signals detected by the DIC-TLM ($\bar{N}$=1).