

## Supporting information

### Ball-lens assisted sensitivity improvement of fluorescence immuno-assay in microchannel

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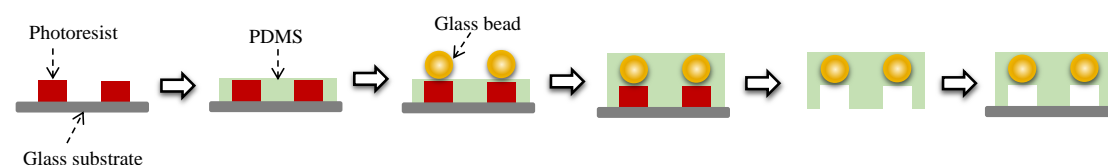
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**Fig. S1** Modified process flow of microdevice fabrication.

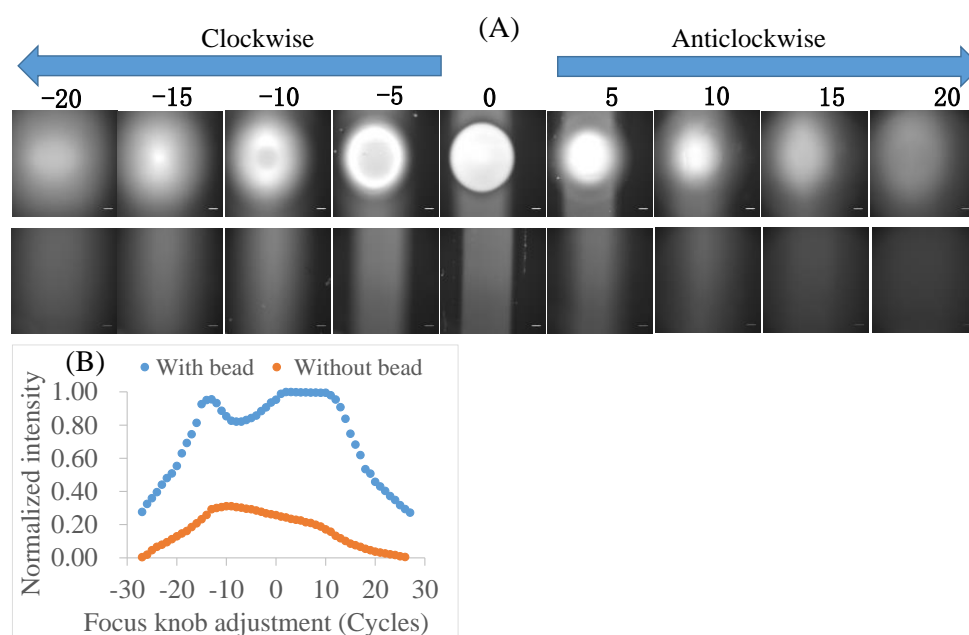
**Fig. S2** The focusing process of the ball-lens and the microchannel.

**Fig. S3** Simulation of ball-lens function using ZEMAX software.

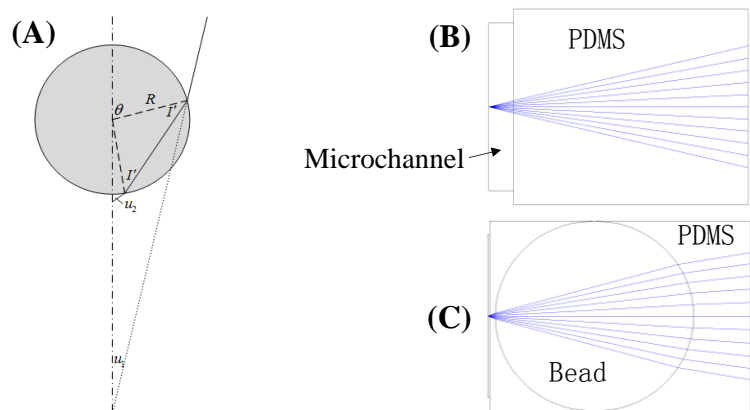
**Tab. S1** The review of CEA analysis in the microfluidic devices.



**Fig. S1.** Modified process flow of microdevice fabrication.



**Fig. S2.** The focusing process of the ball-lens and the microchannel. (A) The original images in the absence of the bead and bead-enhanced images. (B) Plots of normalized intensity against focusing adjusting cycles. ‘0’ represent the best focusing position. The other numbers represent the adjusting cycles of focusing knob from the best focusing position. Positive and negative represent rotational direction.



**Fig. S3.** Simulation of ball-lens function using ZEMAX software. (A) Light-ray tracing of photons emitted from point source undergoing lensing through a ball-lens. (B) Simulation result without the ball-lens. (C) Simulation result with the ball-lens. Simulation parameters: bead diameter is  $700\ \mu\text{m}$  ( $n=1.9$ ). The surrounding medium of the bead is PDMS ( $n=1.45-1.46$ ). The channel width is  $600\ \mu\text{m}$  and filled with fluorescent dye solute ( $n=1.33$ ). The PDMS membrane thickness between the bead and the microchannel is  $\sim 20\ \mu\text{m}$ . The numerical aperture (NA) of  $10\times$  objective is 0.3.

**Table. S1** The review of CEA analysis in the microfluidic devices.

Number	Biomarker	LOD	Labelling	Detection	Reference
1	CEA	0.068 ng/mL	Fluorescein amidite	Fluorescence	B. Li, et al. Clinica Chimica Acta, 2015, 450, 304-309.
2	CEA	0.19 ng/mL	Quantum dots	Fluorescence	L. Liu, et al. Biosensors and Bioelectronics, 2016, 80, 300-306
3	CEA	0.03 ng/mL	Colloidal gold	Thermal lens microscope	K. Sato, et al. Analytical Chemistry, 2001, 73, 1213-1218.
4	CEA	0.19 ng/mL	IRDye800	Near-infrared fluorescence	B. Liu, et al. Advanced Functional Materials, 2016, 26, 7994-8002.
5	CEA	0.41 ng/mL	Alkaline phosphatase	Chemiluminescent	W. Wei, et al. Anal Bioanal Chem, 2011, 401, 3269-3274
6	CEA	5.0 ng/mL	No	Plasmonic wavelength shift	J. Zhou, et al. Nanophotonics, 2019, 8, 307-316.