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

Highly Parallel, Wash-free, and Ultrasensitive Centrifugal Droplet Digital Protein Detection in Sub-Microliter Blood

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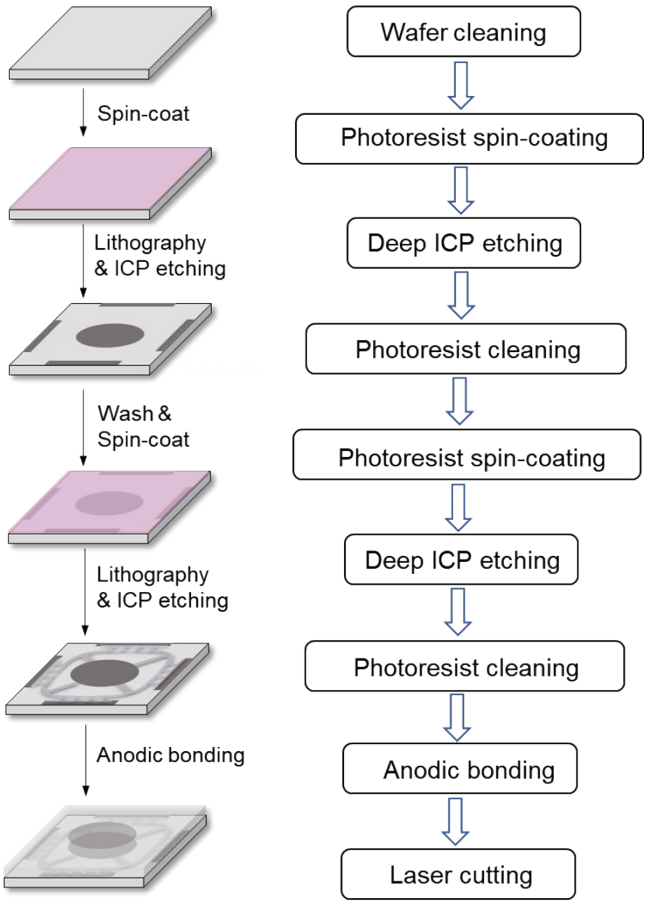

Fig. S1 Schematic of the consecutive two-step lithography-etching procedure for the μCA chip fabrication. 200 μm depth etching was first employed in the center (main channel) and four sides (wells) of a square silicon substrate (dark grey zones). Then, in the second lithography, 4 tributary channels and 16 parallel droplet generators were etched with a depth of 18 microns (light grey zones). After etching, the silicon substrate was permanently bonded with a glass to seal the microchannels. The bonding glass contains a through-hole which can be inserted with a sample container. Laser cutting was finally used to make the chips into individual ones.

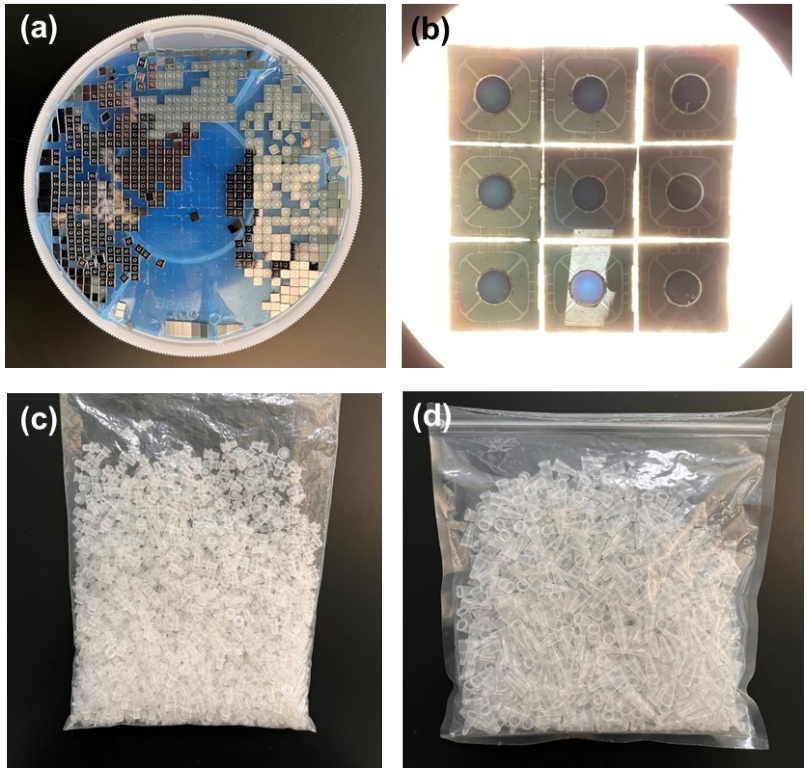

Fig. S2 (a, b) Photos of the laser cutting μCA chips. (c, d) Photos of the plastic sample containers and droplet collectors, respectively. Both the μCA chips and plastic containers can be massively manufactured with high quality.

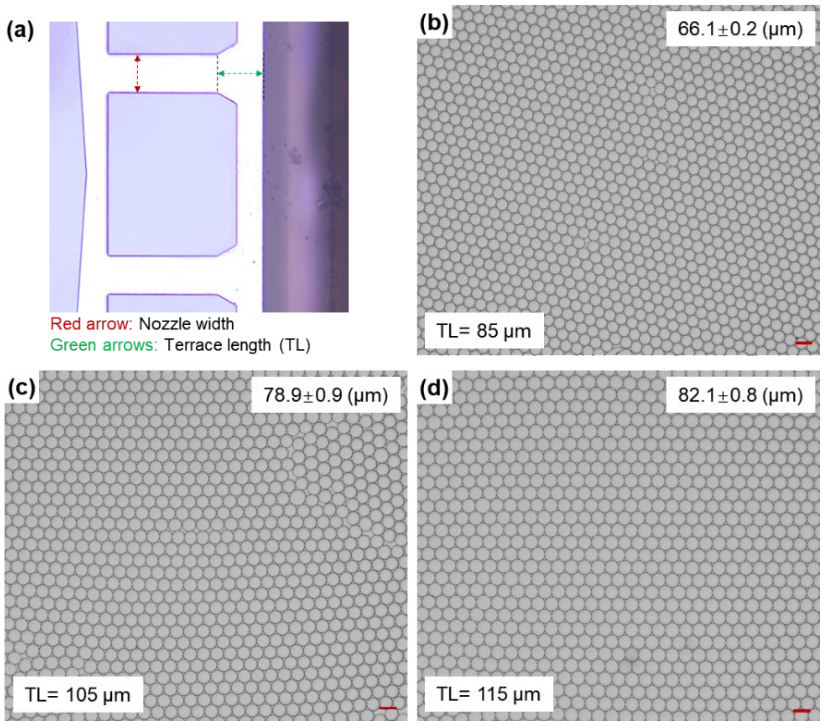

Fig. S3 The effect of the terrace length (TL) of the microchannel on the diameter of the formed droplets. (a) Image showing the nozzle width and terrace length of the microchannel. (b-d) Micrographs of the droplets generated by the chips with different TL. Scale bars: 100 μm.

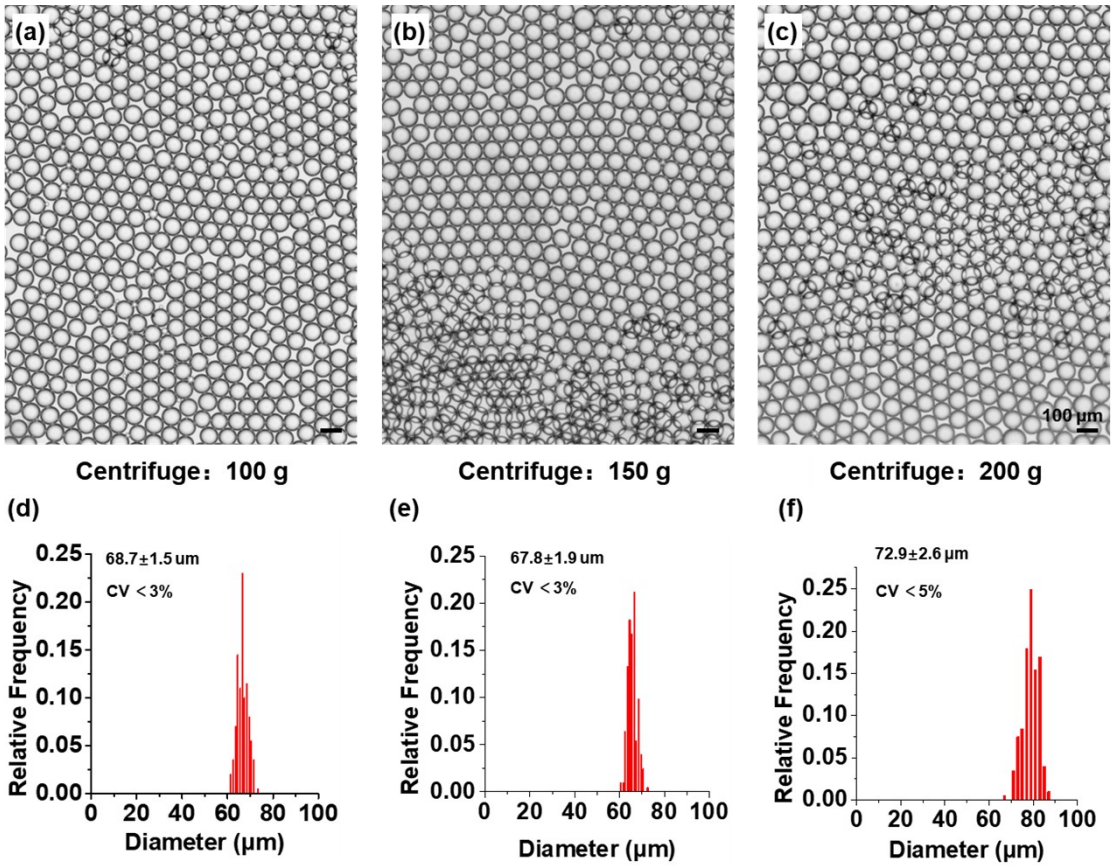

Fig. S4 (a-c) Images and (d-f) the diameter distributions of monodisperse droplets generated at various centrifugal forces of 100 g, 150 g, and 200 g, respectively.

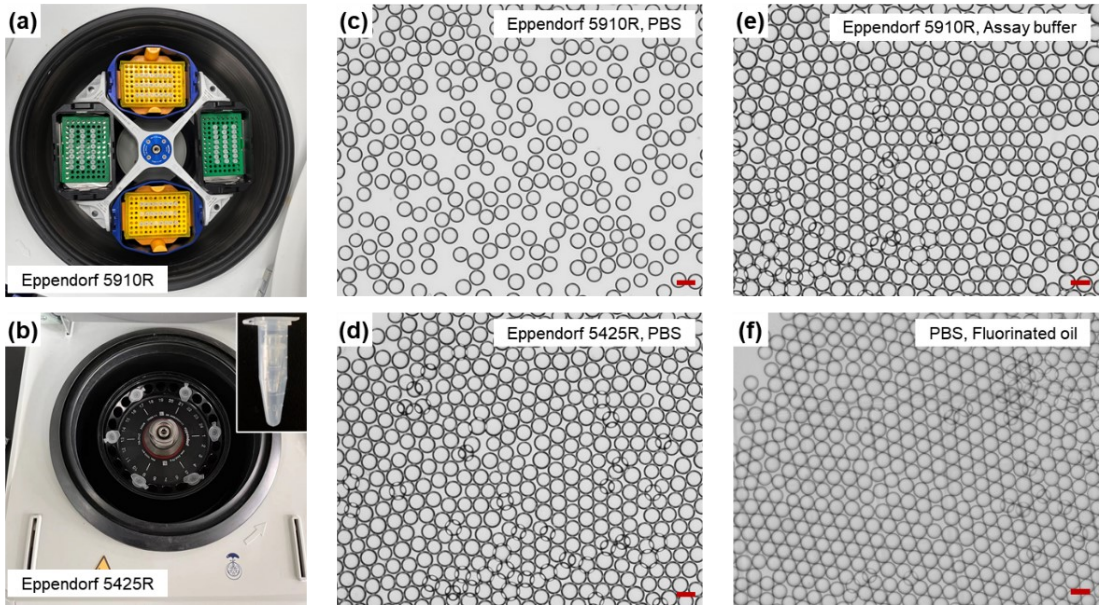

Fig. S5 The effects of the centrifuge types and water/oil/surfactant system on the droplet generation. (a, b) Photos show that two common types of centrifuges were implemented into the droplet generation. (c-e) Microscopy images of the droplets generated with different centrifuges and aqueous solution when mineral oil was used as the continuous phase. (f) Micrograph of the droplets generated when fluorinated oil/surfactant system was employed as the continuous phase. Scale bars: 100 μm.

Description: We first examined the effect of the types of centrifuges on the droplet formation. As shown in Fig. S5a, b, two common types of centrifuges (Eppendorf 5910R and 5425R) were implemented into the droplet generation. The results showed that both centrifuges can generate uniform droplets (Fig. S5c-e). Noted that when Eppendorf 5425R was employed, the device should be placed in a 1.5 mL EP tube before centrifugation (Fig. S5b). The effects of the aqueous solution and continuous oil phase on the droplet formation were also tested. As shown in Fig. S5c-e, the results revealed that the aqueous solution (PBS and assay buffer) has no effect on the droplet formation. In addition, uniform droplets can also be generated when heavy fluorinated oil/surfactant system was employed as the continuous phase (Fig. 53f). These results indicated that our centrifugal device could provide a versatile and flexible platform for droplet generation for common biomedical labs.

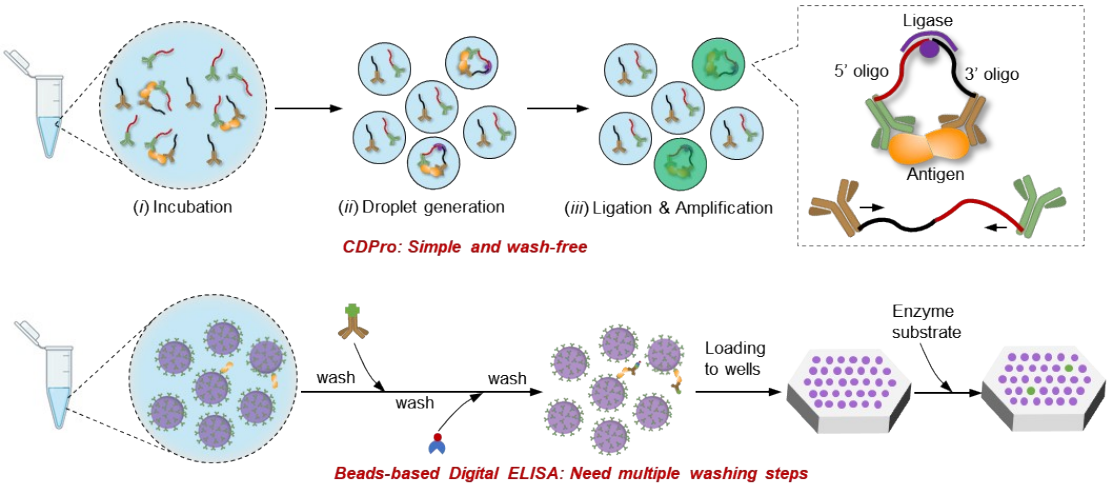

Fig. S6 Schematic comparison of the workflows of the CDPro and bead-based digital ELISAs.

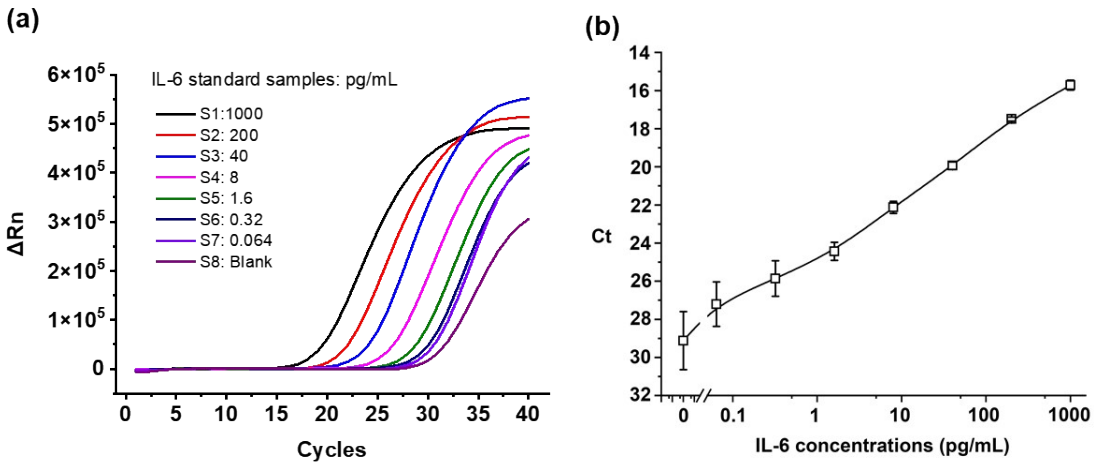

Fig. S7 The qPCR curves and the plot of Ct values *versus* serially diluted IL-6 standard protein concentrations.

Table S1. Cost estimation of the fabrication of ~1000 μCA device[a].

Materials	**Cost (USD $)**	**Average (USD $)**
8-inch silicon	50	0.05
Glass	25	0.025
Mask	150	0.15
Photolithography reagent	120	0.12
Plastic containers	20	0.02
	Total: **365**	Average: ~ **0.365**

[a]About 1000 μCA chips can be fabricated with an 8-inch silicon water.

Table S2. Measurements of target IL-3 and IL-6 in diluted standard samples using CDPro[a].

	S1	S2	S3	S4	S5	S6	S7	S8	Blank
Concentration (pg/mL)[b]	1000	200	40	8	1.6	0.32	0.064	0.0128	0
Copies/μL (IL-3)	/	9120.3	1887.4	447.2	100.2	28.6	14.1	10.5	8.6
Copies/μL (IL-6)	/	9326.4	1740.8	413.7	82.9	26.1	16.6	11.5	8.9

[a]Three repeated expriements were carried out for each sample. [b]Standard samples were 5 times serial dilutied using assay buffer.

Table S3. Measurements of target IL-6 in clinical plasma samples.

	C1	C2	C3	C4	C5	C6	C7
Beckman Dxflex (pg/mL)[a]	1677.17	48.51	35.01	18.33	8.94	1.89	0.93
CDPro (copies/μL)[b]	5028.59	326.61	151.07	92.52	67.68	29.87	13.12

[a]The concentrations of IL-6 in clinical plsama sample were measured by the Beckman Dxflex. [b]The CDPro data of the same samples were obtained from CDPro. The plasma was 10-fold diluted before testing.