Supporting Information

High-throughput Immunoassay through In-channel Microfluidic Patterning

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Experimental Section

Fabrication of micro-immunoassay chip. We fabricated micro-immunoassay chips using multi-layer soft lithography technology. In brief, we fabricated the molds for the fluidic channels and control channels through photolithography. The mold of the control channels was made from 20 μm-thick negative photoresist (SU8-2010, MicroChem, Newton, MA, USA) patterned on a silicon wafer. The mold of fluidic channels was made from positive photoresist (P4620, AZ Electronic Materials, Branchburg, NJ, USA). The fluidic channel was rounded after reflow and its height was about 14 μm. We used PDMS (Sylgard 184, Dow Corning, Michigan, USA) to fabricate the chips. All molds were exposed to chlorotrimethylsilane (TMCS) vapor for 10 min before PDMS casting. The control layer was made from the PDMS with component ratio of 5:1, while the fluidic layer was made of the PDMS with component ratio 23:1. These two layers were bonded together by baking at 80 °C for 30 min in an oven. The assembled two-layer structure was finally placed on an epoxide glass slide (CEL, Arrayit, USA) and then baked in an oven at 80 °C overnight to strengthen bonding.

Materials. Human IgG, Goat anti Human IgG labeled with FITC, polyclonal rabbit anti Human IgG, monoclonal mouse anti Human IgG and polyclonal rabbit anti Human CEA were purchased from Bioscience. Tris-base, BSA and PEG2000 were obtained from Sigma. Monoclonal mouse anti Human CEA and Human CEA standard sample were purchased from Fizrald.

Condition optimization. When we identified blocking buffers' effect to the background signal, we treated each channels with different blocking buffer in parallel using a single chip. After incubating for 10 min, we washed those channels thoroughly with PBS, and then filled the channels with FITC-labeled goat anti-human IgG and incubated for 15 min. After another PBS washing step, we captured the fluorescence images of the channels for analysis. In this process we did not press the buttons for any assays.

Sample preparation and immunoassays. We tested the patient sera in 2X serial dilution using PBS and found that when the sample is diluted once (1:1) the signal-to-noise ratio was the best. We introduced PBS with 1% BSA and 10 mM Tris buffered saline into the microchannels as the blocking buffer, and incubated for 10 min at 25 ^{o}C to block the non-reactive area. We then washed all the channels with PBS for 3 min while kept the button activated. We then released the button valves to expose the unprotected patterns,

and filled the channels with 500 μg ml^{-1} capture antibody, incubated for 15 min at 25 ^{o}C. After the reaction, we washed the channel again with PBS buffer for 3 min. The samples were then loaded into the microchannels and incubated for 15 min at 25 ^{o}C. We later washed the channels with PBS for 3 min. Finally, we filled the channels with 125 μg ml^{-1} detecting antibody and incubated for 10 min at 25 ^{o}C. After PBS washing for 3 min, we took fluorescence images for quantitative analysis.

Image acquisition. An Olympus BX51 upright fluorescence microscope was used for observing and a TuSen CCD (TCC-1.4HICE) was used to capture images. All data was analyzed using Image J and MATLAB.

Table S1. The relationship between the concentration of capture antibody and the detection limit of antigen using sandwich ELISA

Capture antibody concentration (μg ml^{-1})	Antigen detection limit (ng ml^{-1})
0~31.25	>20
62.5	2~20
125	≥0.02
250~1000	≤0.02

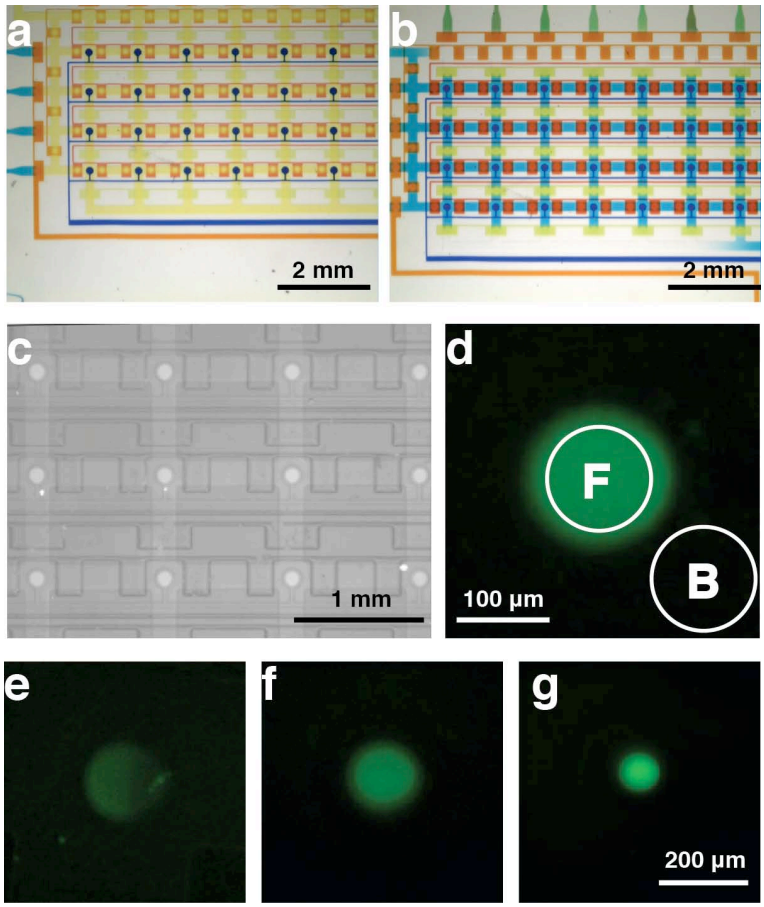

Figure S1. (a) The illustration of blocking process. The blue button valve (blue circle) is activated to protect reaction area. Blocking buffer (yellow solution) cannot access these spots. (b) The illustration of antigen-antibody binding step. The button valve is inactivated and reaction is carried out on the capture-antibody-patterned spots. (c) The array of fluorescent spots from fluorescence ELISA reactions. (d) The image processing of each reaction chamber. The central part of the fluorescent area is cropped with a round-shape mask as the signal, and the non-reaction area is also cropped with the mask of same size for background subtraction. The intensity of each spot was determined by integrating all pixels inside the circle, to minimize the error generated from image noise. (e) The pear-shape fluorescence pattern when button is over-pressurized. (f) The properly pressurized button valve generates a perfect circular reaction area. (g) When the button valve is under-pressurized, the spot size will be reduced. Scale bar is 200 µm.

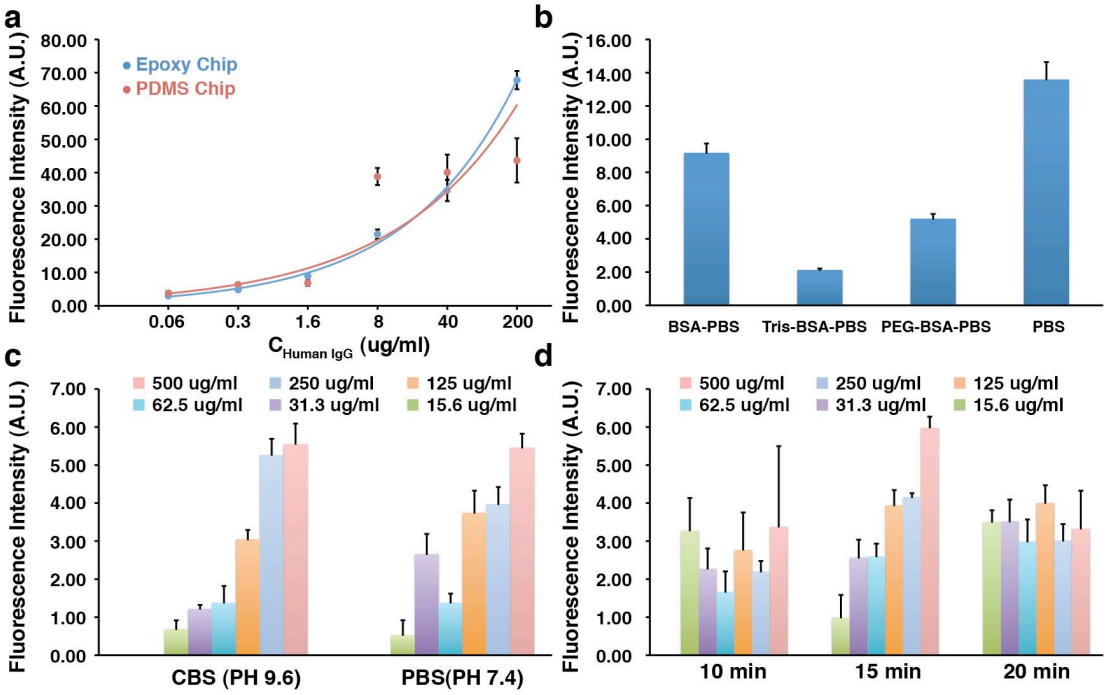

Figure S2. (a) The signal intensity – antigen concentration working curves obtained from the identical designed chips with different substrate. The fitting curve (blue line) for epoxide glass substrate has a high R-square value, 0.99, while the fitting curve (red line) for PDMS substrate has a low R-square value, 0.87. (b) The effect of different blocking buffer. The blocking buffer containing 1% BSA and 10 mM Tris buffered saline shows the best performance. (c) The effect of reaction/dilution buffer. Antigen diluted with carbonate buffer solution (CBS) appears in a normal rising with concentration increasing. This is probably because CBS is slightly basic and hence facilitate the coupling reactions in the immunoassays. (d) The effect of incubation time for immunoglobulin adsorption. The antigen-antibody conjugation is dynamic. Through the experiments we found that 15-min incubation was a proper choice, and the data showed the correct tendency with different concentrations. If the incubation time is too short or too long, the under- or over-adsorption may introduce the artifacts, leading to false determination of the concentrations and large errors between the replicates.

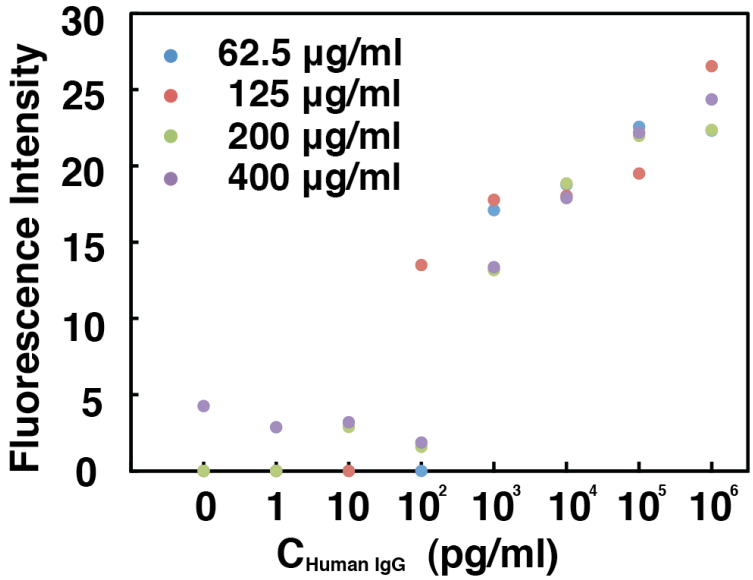

Figure S3. The checkerboard titration for determining the proper concentration of detecting antibody. The capture antibody is 500 µg ml^{-1} mouse anti human IgG monoclonal antibody. The human IgG was diluted from 1 µg ml^{-1} to 1 pg ml^{-1}. The concentrations of detecting antibody are 62.5 µg ml^{-1} (blue), 125 µg ml^{-1} (red), 200 µg ml^{-1} (green), and 400 µg ml^{-1} (purple). During titration we found that when the concentration of Human IgG was too low (10 pg/ml or lower), the intensity difference between the button areas and the background was hard to identify. The data points at this low-concentration range are majorly artifacts, as shown in Fig. S3. In this experiment, 125 µg ml^{-1} detecting antibody has a detection limitation at about 100 pg/ml. With different experimental conditions and different antibodies, this limit may vary.

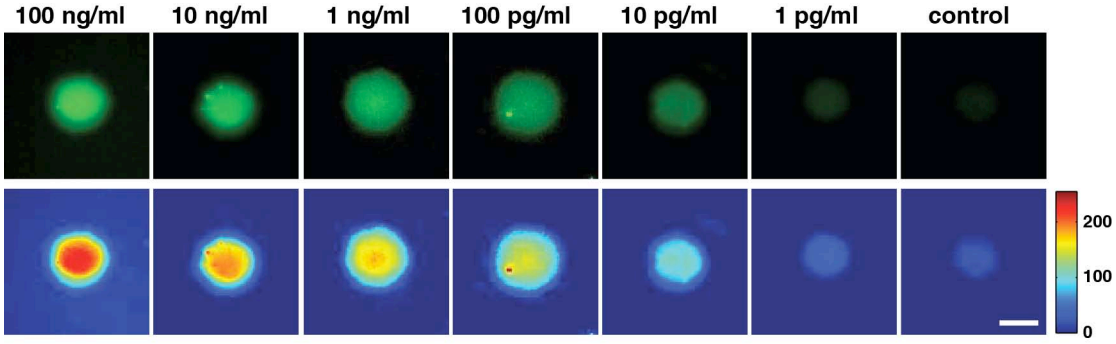

Figure S4. The images of the sandwich ELISA reaction spots with different concentration of human IgG. Capture antibody is 500 μg ml^{-1} mouse anti human IgG monoclonal antibody, and detecting antibody is 125 μg ml^{-1} rabbit anti human IgG polyclonal antibody. The upper row is the original fluorescent pictures and the lower row is the corresponding color maps. Scale bar is 200 μm.

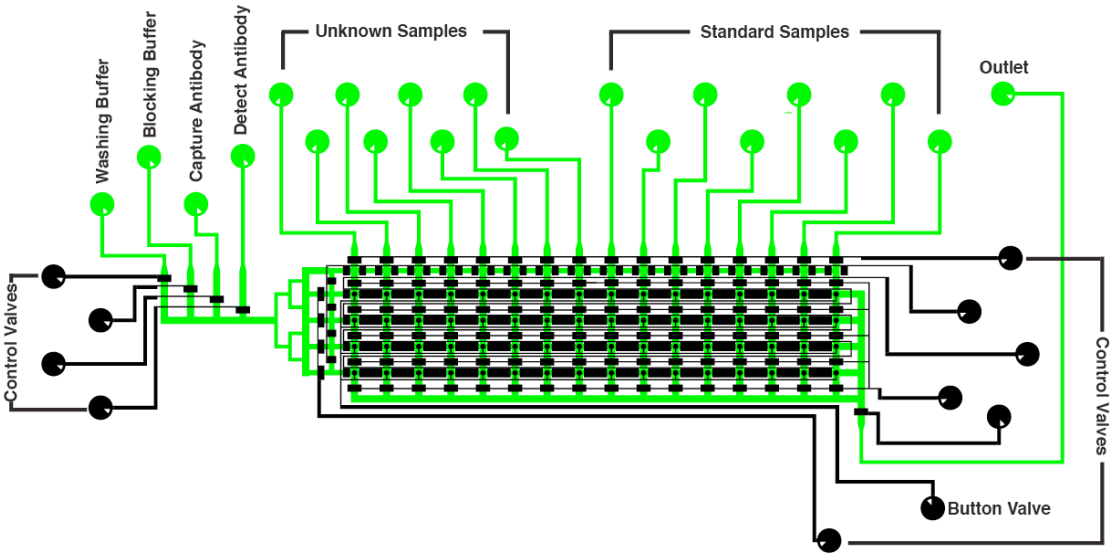

Figure S5. An improved version of microfluidic immunoassay chip. This chip is designed for human CEA detection. The green layer is the fluidic layer, and the black layer is the control layer. The left four inlets are designed for introducing the common reagents, including washing buffer, blocking buffer, capture antibody and detecting antibody. Sixteen inlets on the top part are designed for introducing unknown samples and CEA standard samples.