Supplementary information

Multidimensional analysis of the frequencies and rates of cytokine secretion from single cells by quantitative microengraving

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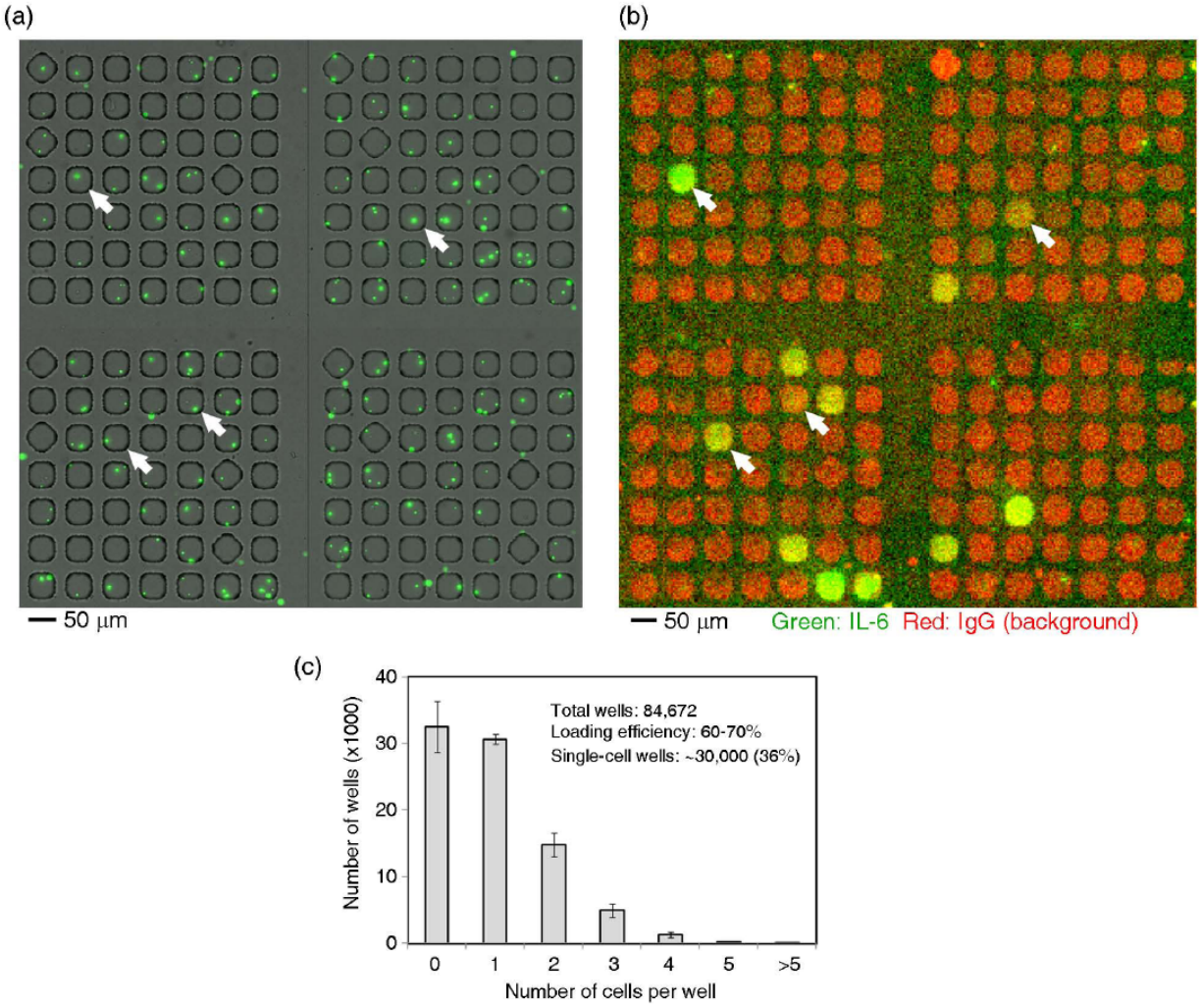

Fig. S1 Loading efficiency of cells in microwells and corresponding secretion profile. Composite micrographs of cells in microwells (a) and their secretion profile captured on glass surface (b). Live cells were stained with Calcein violet AM (indicated by green in (a)) and imaged before microengraving. IL-6 secretion was detected by fluorescent IL-6 antibody (green channel in (b)). Array of wells was highlighted on the glass surface by detecting trace IgG added in the media (red in (b)). Single cells with IL-6 secretion are indicated by arrows in the images. (c) Distribution of the number of cells per well determined from 5 independent experiments. By controlling cell density and loading time, normally ~30,000 single-cell wells can be achieved on one array.

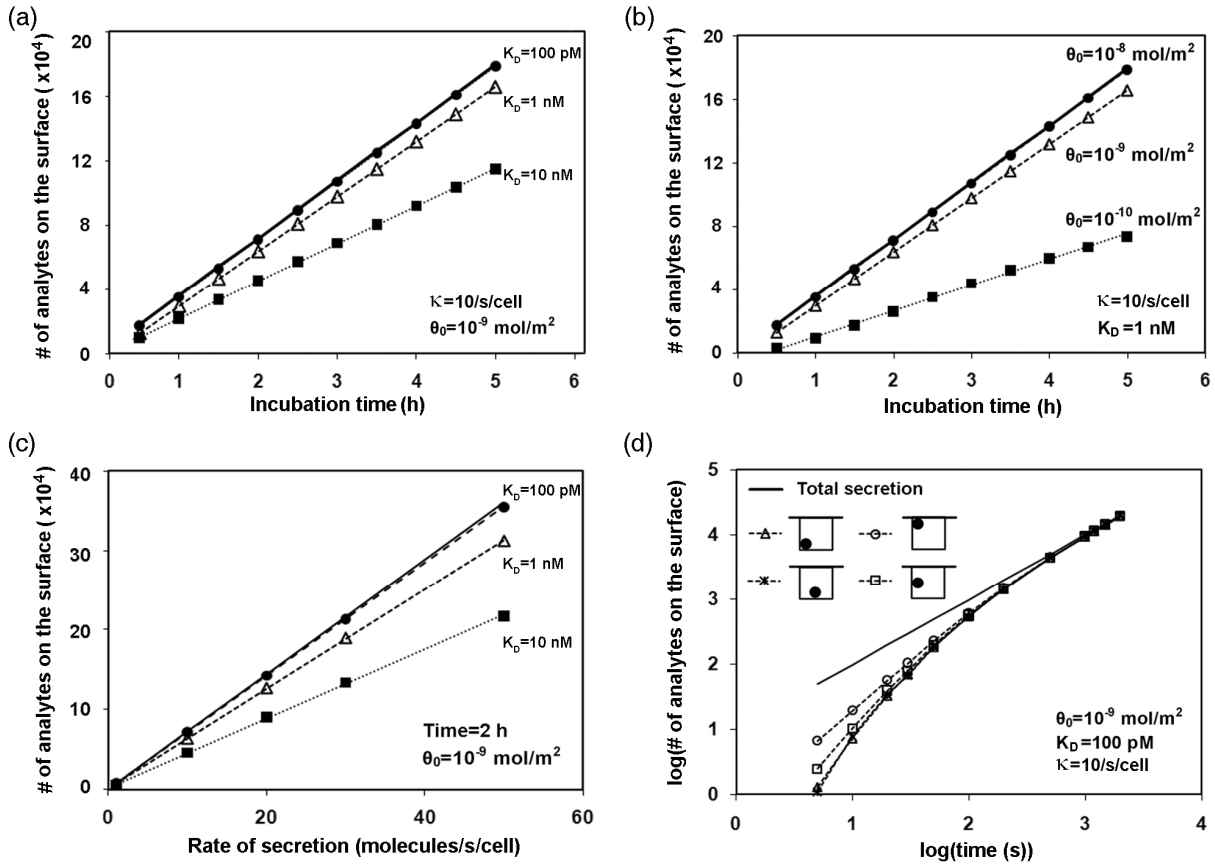

Fig. S2 Effects of model parameters on the calculated number of analytes captured at the surface. (a) Plot of the number of analytes captured as a function of the incubation time for capture antibodies with three different affinities (K_D). (b) Plots of the number of analytes captured as a function of the incubation time for three different densities of binding sites on the surface. (c) Plots of the number of analytes captured as a function of the rate of secretion for capture antibodies with three different K_D. (d) Plots of the number of analytes captured as a function of the incubation time with varied positions of the cell within the microwell (relative to the glass). Solid lines in all four panels represent the total quantity of analytes secreted as a function of time.

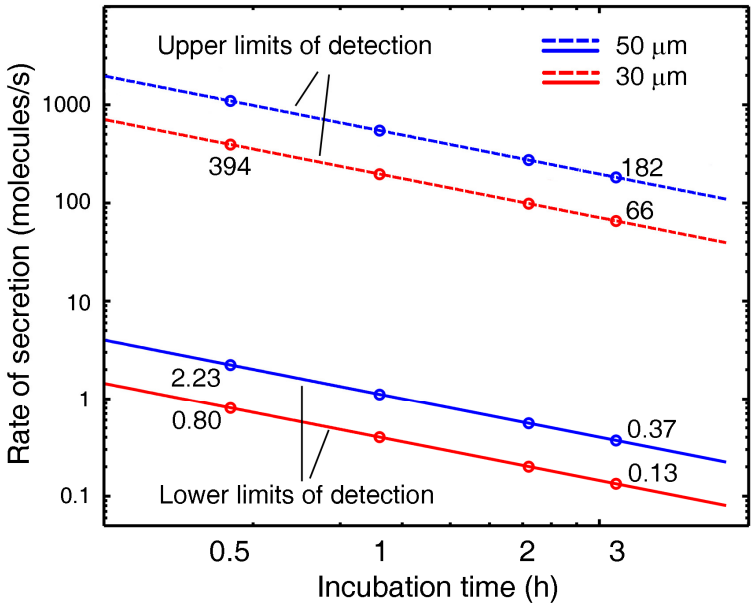

Fig. S3 Optimal windows of cytokine capture in 50 μM (blue) and 30 μM (red) microwells. At each time point, the lower limit of detection is the minimal rate of secretion that provides signal above backgound+3SD. This line is determined experimentally and might vary with different antibody pairs and fluorophores. An average result from IL-2 detection is presented in this graph. Based on the total binding sites on the surface, when each capture antibody only binds one cytokine, the average distance between cytokines is ~36 nm, more than twice of the size of IgG (~15 nm). If captured cytokine is below this density, we assume that the binding between detection antibody and captured cytokine is monovalent, thus allowing a linear estimation of the number of cytokine through the number of detection antibody. We defined the rates of secretion that achieve this density at each time point as our upper limits of linear detection. Region between lower limits and upper limits is the optimal window for quantification. The lower and higher limits of several time points (0.5, 1, 2, and 3 h) are indicated in open circle on the graph along with the corresponding rates of secretion.

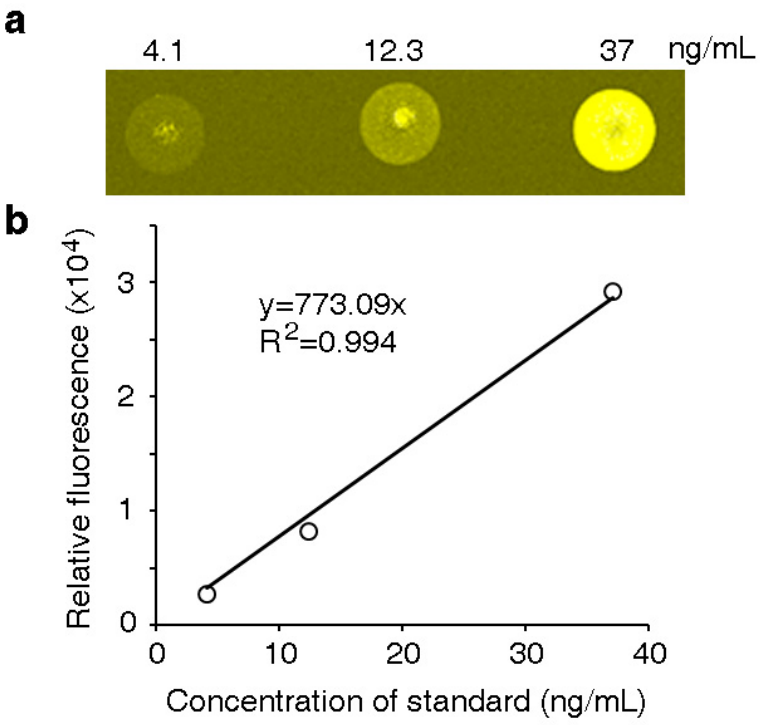

Fig. S4 Micrograph of a standard reference slide (a) and a sample calibration curve (b) used to calculate the rate of secretion from the cells. A series of diluted, fluorescently labeled detection antibody was spotted on the glass (1 μL/spot) at the concentrations indicated, and the mean fluorescence intensity of each spot was plotted to generate the calibration curve (solid line).

Table S1 Distribution of cell types in total PBMCs and IL-6 secreting cells

	% of total PBMCs	% of IL-6 secreting cells
CD3+	32.4	4.7
CD11b+CD14–	23.5	44.8
CD11b+CD14+	5.4	26.9
Others	38.7	23.6