Supporting information

Fluorescence quantification of intracellular materials at the single-cell level by an integrated dual-well array microfluidic device†

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Supplementary Movie Legend
1. Supplementary Movie 1. Dynamic process of generating single-cell array by washing out the cells in the reaction-wells.
2. Supplementary Movie 2. Dynamic process of sealing each reaction-well by oil.

Figure S1. Manufacturing process flow diagram of dual-well array chip. The chip was fabricated with p-type (100) single silicon wafers. (a) Positive photoresist AZ 6130 (thickness 3 μm) was spin-coated onto the wafer using a photolithography mask and the reaction-wells (200 μm diameter) photoresist array were generated (b). (c) Under the protection of photoresist, reaction-well array pattern was formed using deep reactive-ion etching in the depth of 20 μm and the remaining photoresist was removed with acetone. (d) The secondary lithography process was used to form capture-well pattern. (e) Positive photoresist AZ 6130 (thickness 3 μm) was spin-coated onto the wafer and capture-well (20 μm diameter)
photoresist array was generated. (f) Using deep reactive-ion etching to manufacture capture-well array in the depth of 20 μm and removing the photoresist (g).

Figure S2. Merged fluorescence micrograph of K562 cells in dual-wells labeled with Hoechst dye. The fluorescence image was inverted into grayscale images. The white spots stand for pre-stained K562 cells. Supplementary results of single-cell capturing experiments, A, B, C and D. For experiment A, the occupancy of single-cell is 55.7% and multi-cell is 2.2%.

Figure S3. (a) Fluorescence images of Alexa Fluor 488 dye regent sealed in the dual-wells. The relative
standard deviation (RSD) of the fluorescence intensity was 2.1%. (b) The stability of droplet was also evaluated by calculated the fluorescence difference along saving 0, 6, 12 and 24 hours. (b)-(d) The K562 cells were centrifuged at 2000 rpm with 2 minutes on a silicon substrate stained both Hoechst and Propidium iodide solution and (e)-(f) on the dual-well chip.

Figure S4. Original fluorescent images of enzymatic assay of K562 cells using dual-well array chip. The total number of dual-wells we collected was 146.

Figure S5. (a) Original fluorescent images of 0, 1, 5, 10µM D-glucose. (b) Original fluorescent images of stained K562 cells in the dual-wells and corresponding fluorescence from intracellular glucose. The total number of dual-wells we collected was 107.