Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Trapping cells on a stretchable microwell array for single-cell analysis

Yuli Wang, Pavak Shah, Colleen Phillips, Christopher E. Sims, Nancy L. Allbritton

1. Stretchable microwell arrays and the stretching device, and detailed protocol for cell trapping and cell staining

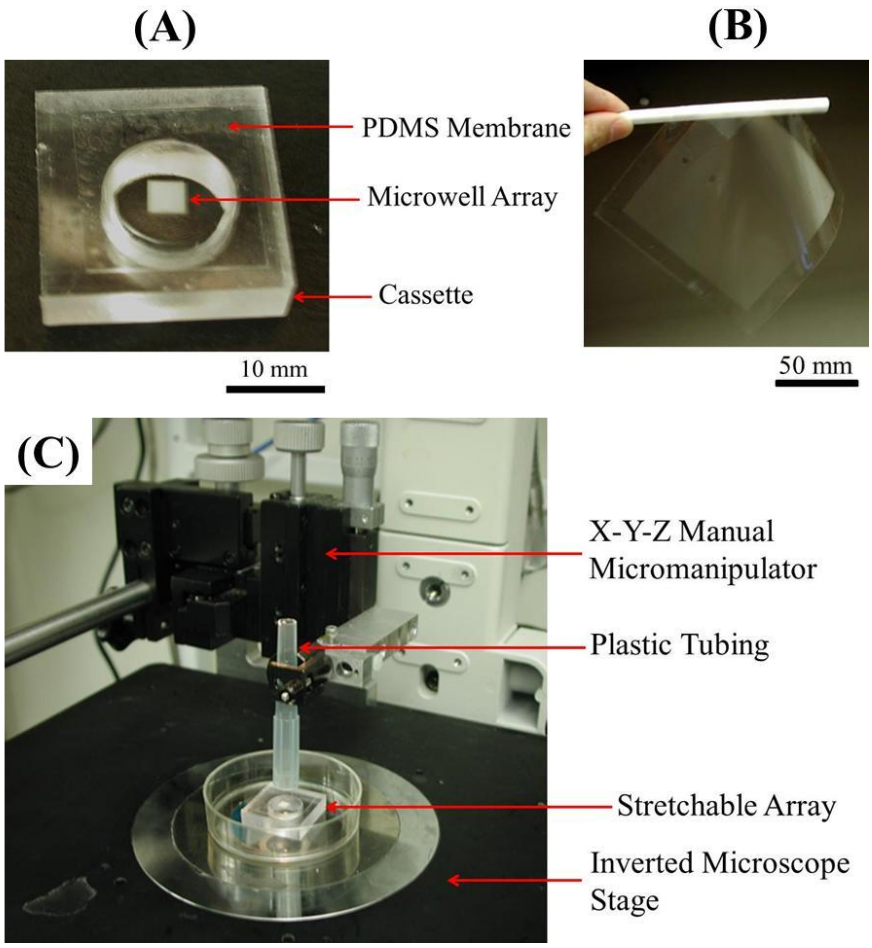


Fig. S1. Photos of stretchable microwell arrays and the stretching device. (A) A PDMS stretchable microwell array used in the current study. The array had a dimension of 3 mm × 3 mm and was attached to a rigid cassette. (B) An extra-large PDMS microwell array fabricated by micromolding against a master. The array has a dimension of 100 mm × 100 mm. (C) Setup of the stretching device. A plastic tubing was attached to a X-Y-Z manual micromanipulator. The

stretching of PDMS microwell was controlled by the Z-axial movement of the tubing. The extent of stretch and diameter of microwells can be monitored by microscope.

A simple stretching device was built to reversibly, uniaxially stretch the flexible PDMS microwell array prior to cell loading (Fig. S1C). A manual micromanipulator (World Precision Instrument, model# M3301R) was mounted on an inverted microscope (Nikon Eclipse TE300). A plastic tube (diameter: 7 mm, length: 50 mm) cut from a 1 mL polypropylene pipet tip was attached to the micromanipulator. The tube was used to stretch the microwell array by applying circumferential pressure on the array in a downward direction as described below. The PDMS microwell array was treated in an air-plasma cleaner for 30 s to render its surface hydrophilic. The array was placed on the microscope stage and stretched by depressing the PDMS membrane with the plastic tube. Typically, 6 mm of z-axis displacement of the tube against the PDMS membrane surrounding the microwell array expanded the individual microwells from 12 μm to 20 μm in diameter. Then 1 mL of a suspension of Ba/F3 cells (1.25×10^6 cells/mL) in medium was added to the plastic tubing, and the cells were allowed to settle for 5 min to load the microwells. The microscope stage was gently tapped with a finger at ~ 1 Hz to assist the cells settling into the microwells. The plastic tube was then slowly raised at a speed of ~ 2 mm/min to release tension on the PDMS membrane. After the extraction of the plastic tubing, the superfluous cell suspension was aspirate by a glass pipette to a waste container via vacuum. Cells became trapped in the microwells as the flexible PDMS membrane relaxed to its original dimensions. To remove non-trapped cells, the cassette housing the array was tilted and the chamber was rinsed 5× with 1 mL of PBS buffer.

When performing assays in the device, the reagents can be directly added to the array. Since the array is small (3 mm x 3 mm), it only requires about 100 µL reagent to stain all cells trapped on the array. After staining, the unbound reagent was removed by rinse the entire array by PBS.

2. Proliferation of trapped Ba/F3 cells

Cells trapped on the PDMS microwell array experience compression with some deformation of their cell bodies. To demonstrate that cells were able to remain viable for a prolonged period of time after entrapment, Ba/F3 cells were trapped on the array and imaged at 0 h (Fig. S2A) and 20 h (Fig. S2B). Not only were the cells viable, but they were able to proliferate as well. Their daughter cells can migrate out of the traps.

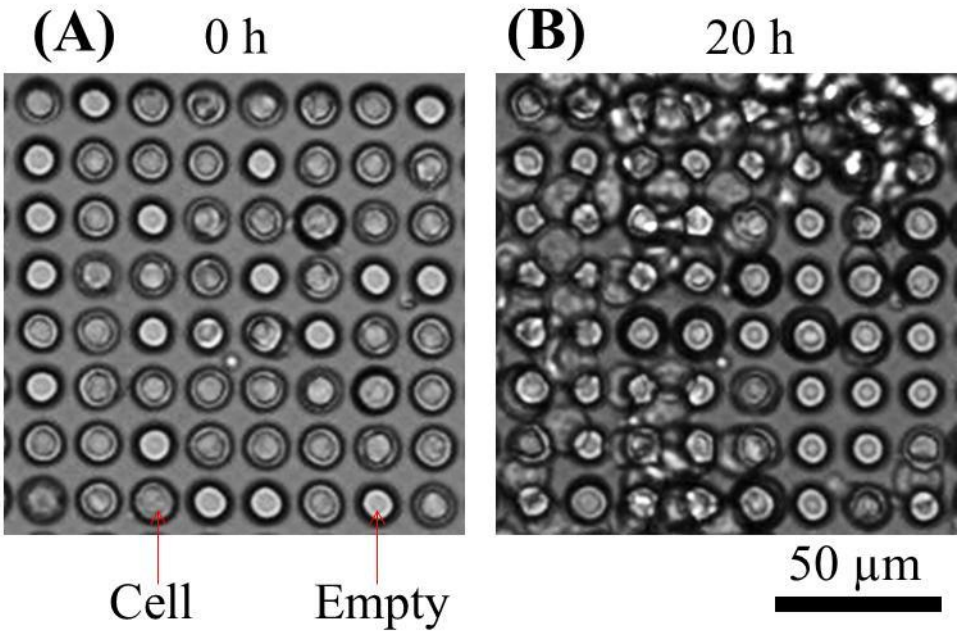


Fig. S2. Proliferation of trapped Ba/F3 cells. Ba/F3 cells were trapped on the microwell array. Transmitted light images were taken at (A) 0 h and (B) 20 h.

3. Comparison of the stretchable microarray and regular microwell array for cell retention after rinsing

The trapping was seen to be relatively stable on the stretchable array, permitting fluid exchange on the array without loss of trapped cells. To quantify the stability of cell trapping, the post-rinse cell retention was compared on stretchable microwell array and on a standard microwell array formed in PDMS and of similar dimensions. During rinse, the cassette housing the array was tilted and the chamber was rinsed ×5 with 1 mL of PBS buffer using a pipette.

On the stretchable microwell array (diameter: 12 μm, height: 15 μm), a suspension of 323,000 cell/cm^2 was used. The trapped cells were stained with Oregon Green 488 carboxylic acid diacetate and rinsed with PBS ×5. The array was then imaged before and after a second series of rinses (×5) with PBS. It was found that 99.5 ± 0.3% of the cells were retained after the second series of rinses (n = 5 images, 4,007 cells counted).

On the standard PDMS microwell array, the dimension of the microwells must be slightly larger than that of the cells for optimal cell capture efficiency. A microwell array with a diameter of 20 μm and a height of 15 μm was found to be appropriate for efficiently capturing the Ba/F3 cells. Due to a high rate of cell loss in carrying out a standard staining protocol on cells already in the microwell array, cells were pre-stained with the Oregon Green 488 dye and loaded on the array. The same cell density of 323,000 cell/cm^2 was used to load the microwell array. The array was rinsed with PBS ×5 to remove cells that did not reside in the wells. The array was then imaged before and after a second series of rinses (×5) with PBS to parallel the procedure used for the stretchable microwell array. Only 69 ± 19% cells were retained on the array after the second series of rinses (n = 5 images, 1,465 cells counted).

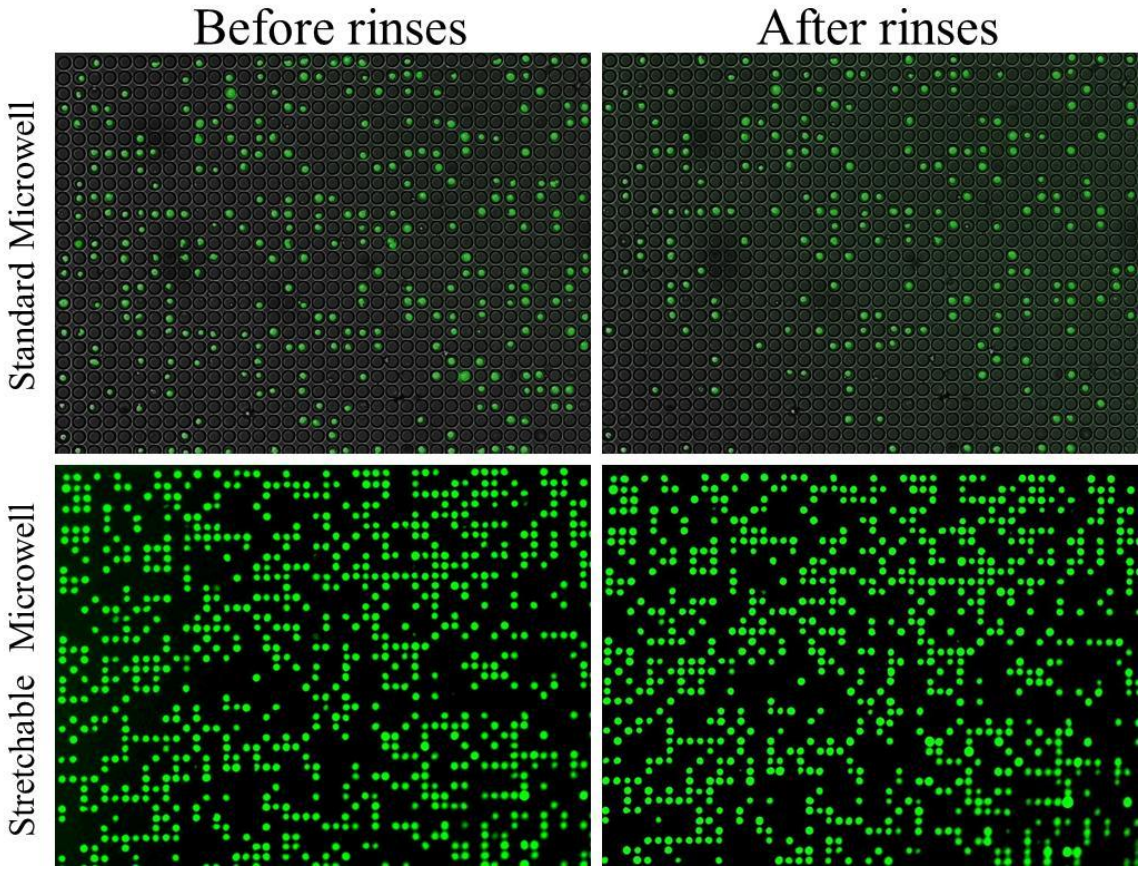


Fig. S3. Cell retention after rinsing for the stretchable microarray and regular microwell array.

4. Variables affecting cell trapping efficiency on the array

Ba/F3 cells have an average diameter of 14.1 μm. The ideal cell trap would have a diameter of slightly smaller than 14.1 μm, and a height slightly larger than 14 μm, so that cells can be effectively trapped without experiencing too much compression. To achieve the optimal trapping efficiency (defined as the percentage of wells that can capture cells), we studied the effect of a variety of factors such as cell loading density, Z-axis stretching displacement, and height of the microwells. The optimal density for cell loading on the array was 1.25×10^6 cells/cm^2 (Fig. S4A), the stretching z-axis displacement was 6 mm (Fig. S4B), the microwell height was 15 μm (Fig. S4C). The optimal microwell diameter was 10-12 μm. These graphs were obtained with the other

two remaining parameters already optimized. These graphs were replicated with three different chips to obtain the average value and standard deviation.

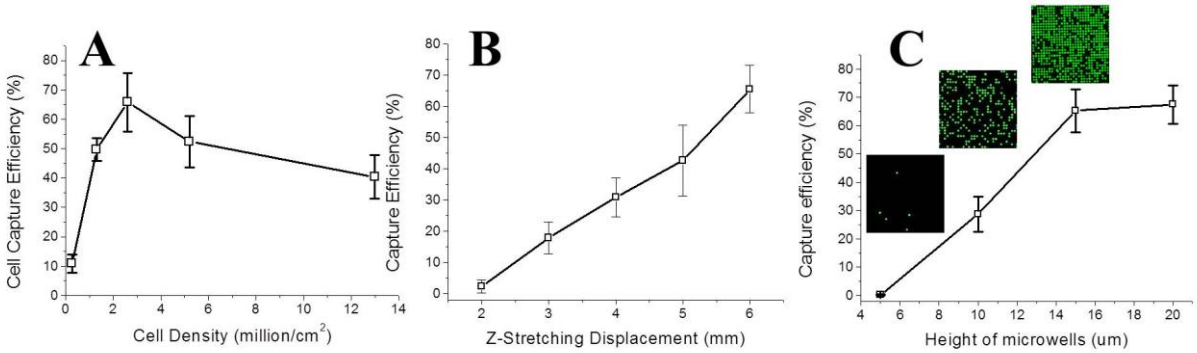
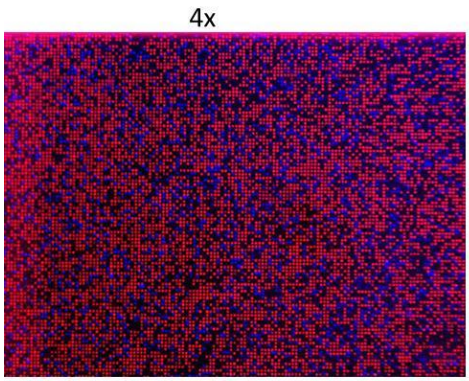
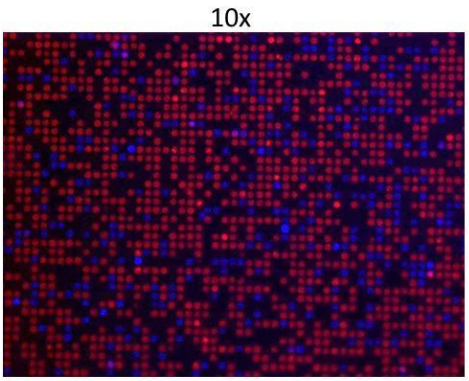


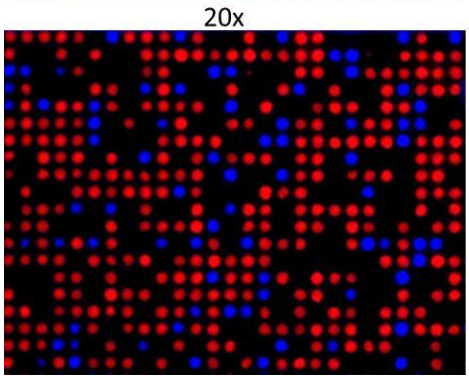
Fig. S4. Variables affecting cell trapping efficiency on the array. (A) Density of the cells for loading the array. (B) Z-axis stretching displacement. (C) Height of the microwells. The inserted fluorescence images show the cells trapped on the array when height of microwells were 5 μm, 10 μm and 15 μm, respectively. The error bars are standard deviation.

5. Two-color, single-cell analysis

To demonstrate multi-color, single-cell analysis, we have tested fluorescence dyes of additional wavelengths, for example CellTracker Blue and Orange. The cells were stained with 10 μM CellTracker Blue CMAC and CellTracker Orange CMTMR separately. These two populations were mixed at an approximate ratio of 4:1 (orange:blue), and the cells were trapped on the array. Fig. S5 shows combined images obtained with different magnification. Two array samples were used to capture a mixed cell population in 3 independent experiments.







	Blue cells	Orange cells	Total cells	Blue cells ratio	Average
Sample 1-1	1823	6429	8252	0.220916	
Sample 1-2	1782	6514	8296	0.214802	0.217859
Sample 2-1	1517	5639	7156	0.21199	
Sample 2-2	1589	5951	7540	0.210743	0.211367
Sample 3-1	1601	5600	7201	0.22233	
Sample 3-2	1643	5850	7493	0.219271	0.220801

21.67±0.48% blue cells

Fig. S5. Two-color single-cell analysis using CellTracker Blue and Orange. Images were obtained at varying magnification from 4-20×.