Supporting Information for *Intuitive, Image-Based Cell Sorting Using Opto-fluidic Cell Sorting*

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Table S-1. Laser Parameters at Onset of Cell Damage from Literature

Ref.	Cell Type	λ (nm)	Power (W)	Exposure Time (s)	Spot Size (μm)	Power Density (W/cm^2)	Energy Density (J/cm^2)	Energy (J)	Damage
Liang[20]	CHO	990	0.176	180	0.70	4.6e+7	8.2e+9	31.7	Clonability
Liu[22]	Human Sperm	1064	0.300	120	0.75	6.8e+7	8.1e+9	36.0	Viability (propidium iodide)
Mohanty[23]	NC37 Lymphoblast	1064	0.120	30	0.75	2.7e+7	8.1e+8	3.6	DNA damage
Wang[24]	HeLa	1070	13.2	0.004	4.9	7.0e+7	2.8e+5	0.053	None
Our Device	BA/F3	980	0.125	20	8.6	2.2e+5	4.3e+6	2.5	

Table S-1 is an adaptation of a supplemental table from Wang *et al.* with some reference to original literature to use more relevant data points for comparison with our experiments. We report our spot size as the spot diameter measured across the beam between points of $1/e^2$ of maximum spot intensity as measured with an unsaturated CCD. Spot sizes listed for comparison are spot diameters calculated by $d=1.22\lambda/(n \times NA)$ according to the cited NA and wavelength in the reference. The operating point of Wang *et al.* was reported to induce no damage and is shown for comparison. Our parameters are gentler than the damage threshold reported by Liang, Liu, and Mohanty, especially with respect to power density and energy density.

Table S-2. Quantification of Device Performance

Before Sorting	Experiment 1	Experiment 2	Experiment 3
Cell Concentration	2.2e6 mL^{-1}	3.0e6	3.0e6
Loading Efficiency	32 %	20	32
# Total Cells	3337 cells	2079	3294
Target Cell Purity	5 %	5	6
Target Cell Ratio	1/19.0	1/19.2	1/14.7
During/After Sorting			
# Removals Attempted	119	92	156
% Removed	66 %	97	82
Target Cell Purity	81 %	89	84
Target Cell Ratio	4.1/1	8.1/1	5.4/1
% Cells Lost	21%	18	28
# Cells In Output	62 target : 15 unwanted	73 : 9	92 : 17
Enrichment	78.4	155.4	79.4

Table S-2 quantifies various aspects of device operation. Cell concentration is the concentration of the cell suspension prior to injection. Loading efficiency is the percentage of trap sites containing at least one cell following device loading. Total cell number refers to the total number of cells residing in microwells after loading. Target cell purity is the percentage of target cells comprising the total cell population. Target cell ratio is the ratio of target cells to unwanted cells. Number of removals attempted refers to the number of target cells that we attempted to remove. Percent removed refers to the percentage of target cells successfully removed in all attempted optical removals. Percent of cells lost is the percentage of target cells unaccounted for between release from the array and arrival at the output reservoir. Number of cells in output states the number of target and unwanted cells which arrived at the output reservoir. Enrichment is the target cell ratio after sorting divided by the target cell ratio before sorting. We intentionally did not remove a fraction of target cells from the array in each experiment in order to have reference particles for unperturbed target cells to more easily determine reasonable fluorescence exposures for reservoir analysis images.

Figure S-1: Enrichment of Target Cell Population

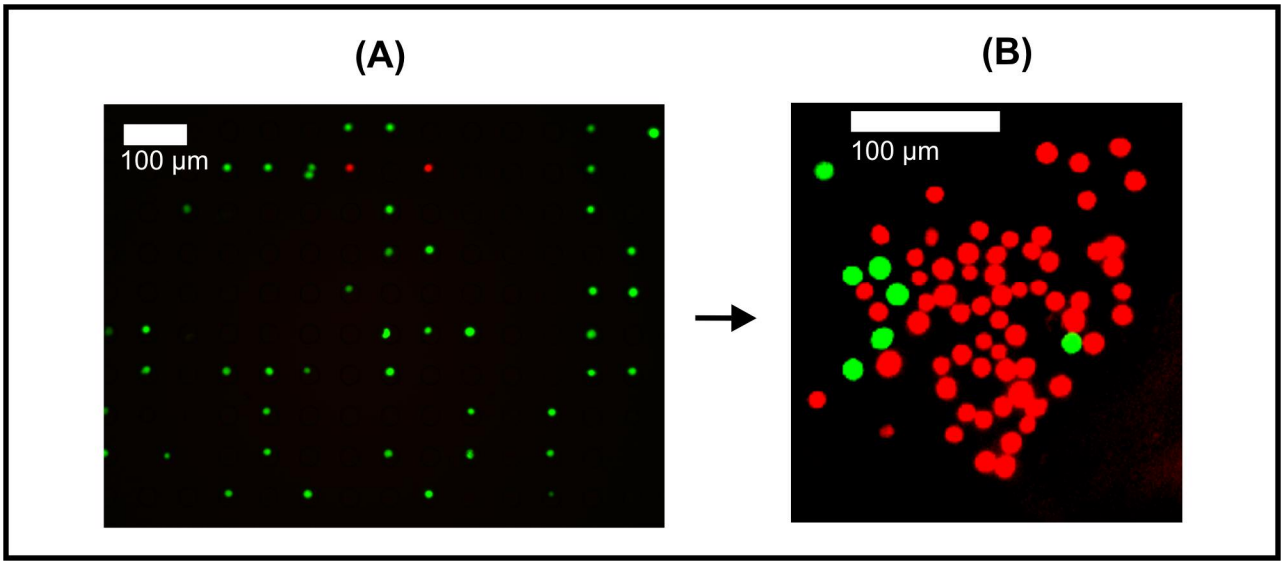

Figure S-1 qualitatively illustrates the enrichment in the 155-fold enrichment case. (A) shows a part of the original cell array with an approximately representative ratio of minority population to majority population cells. (B) is a fluorescence image of part of the bottom of the collection reservoir illustrating part of the sorted population. We used images similar to these to produce the data in Table S-2. Green channel in (A) and red channel in (B) are oversaturated for easier visualization.

Video S-1. Levitation and Release of a Single Cell from a Microwell

Video S-1 shows cells initially resting in a section of a microwell array. We focus the laser (which appears as the bright spot on the cell) onto a particular cell soon after the video begins. The cell moves laterally into the beam center and is subsequently levitated into the flow field of the chamber, where drag eventually carries the cell away. This video is available free of charge at http://pubs.acs.org.

Video S-2. Levitation and Release of a Single Cell from a Double-loaded Microwell

Video S-2 shows cells initially resting in a section of a microwell array. We focus the laser onto a single cell in a doubly-loaded microwell and selectively remove the single cell while leaving behind the untargeted cell. This video is available free of charge at http://pubs.acs.org.