

# Microfluidic Platform for the Isolation of Cancer-Cell Subpopulations based on Single-Cell Glycolysis

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## Supplemental Information

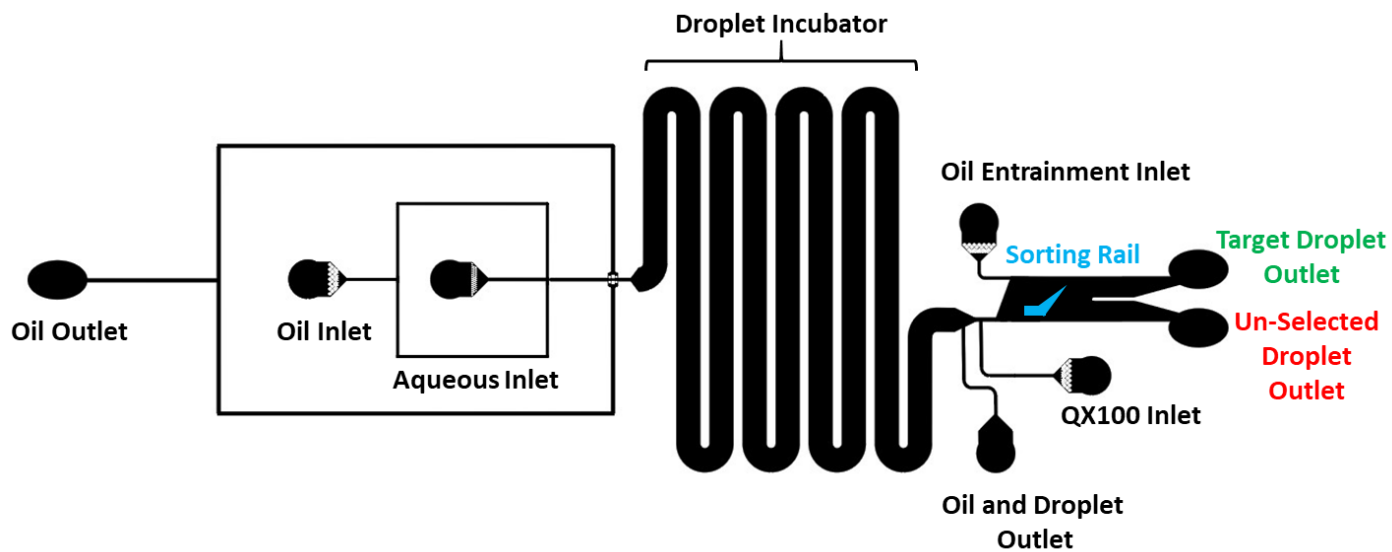
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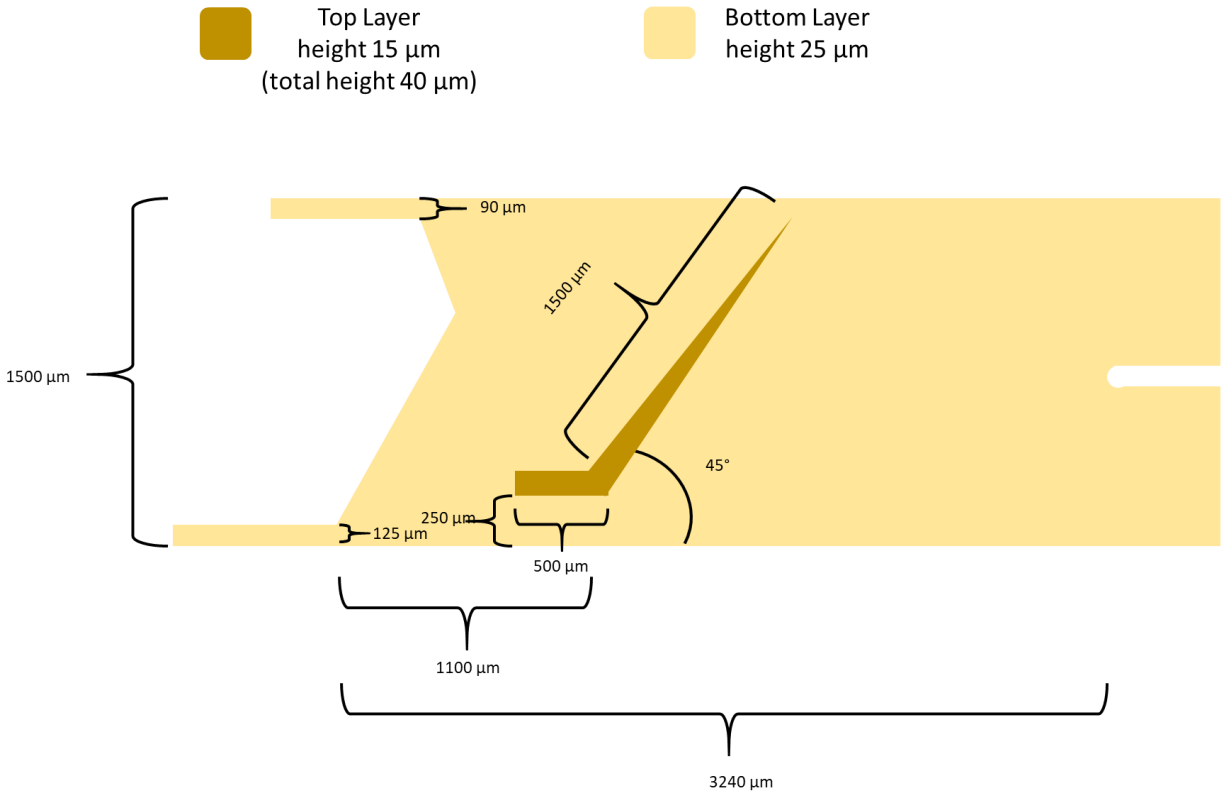
**Video Caption:**

**Video S1.** Selection of droplets of different pH containing K562 cells using SIFT. Video shows the end of incubator and sorting region.

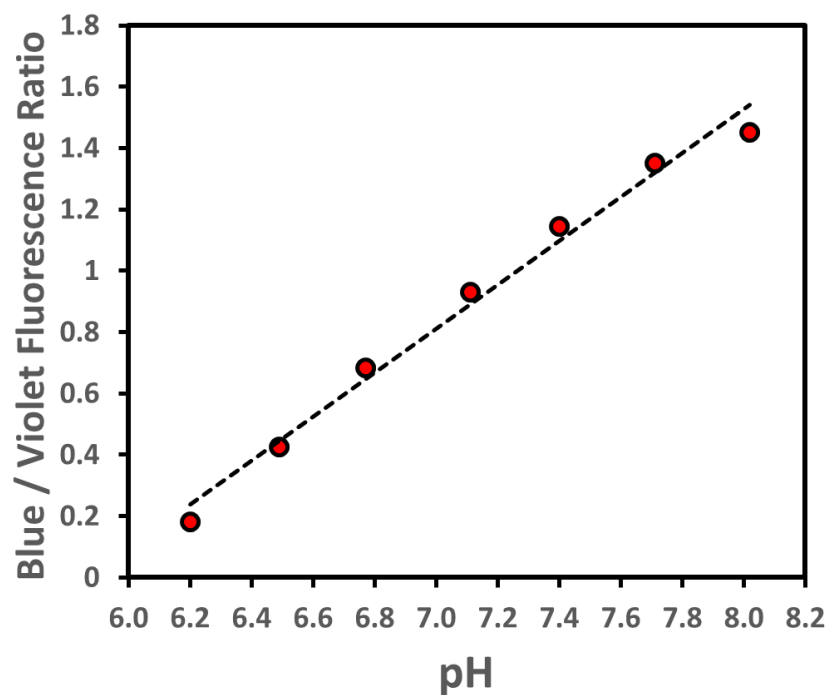
**Video S2.** SIFT device sorting of hypoxia treated and control (normoxia) MDA-MB 231 cells. Video shows the end of incubator and sorting region.



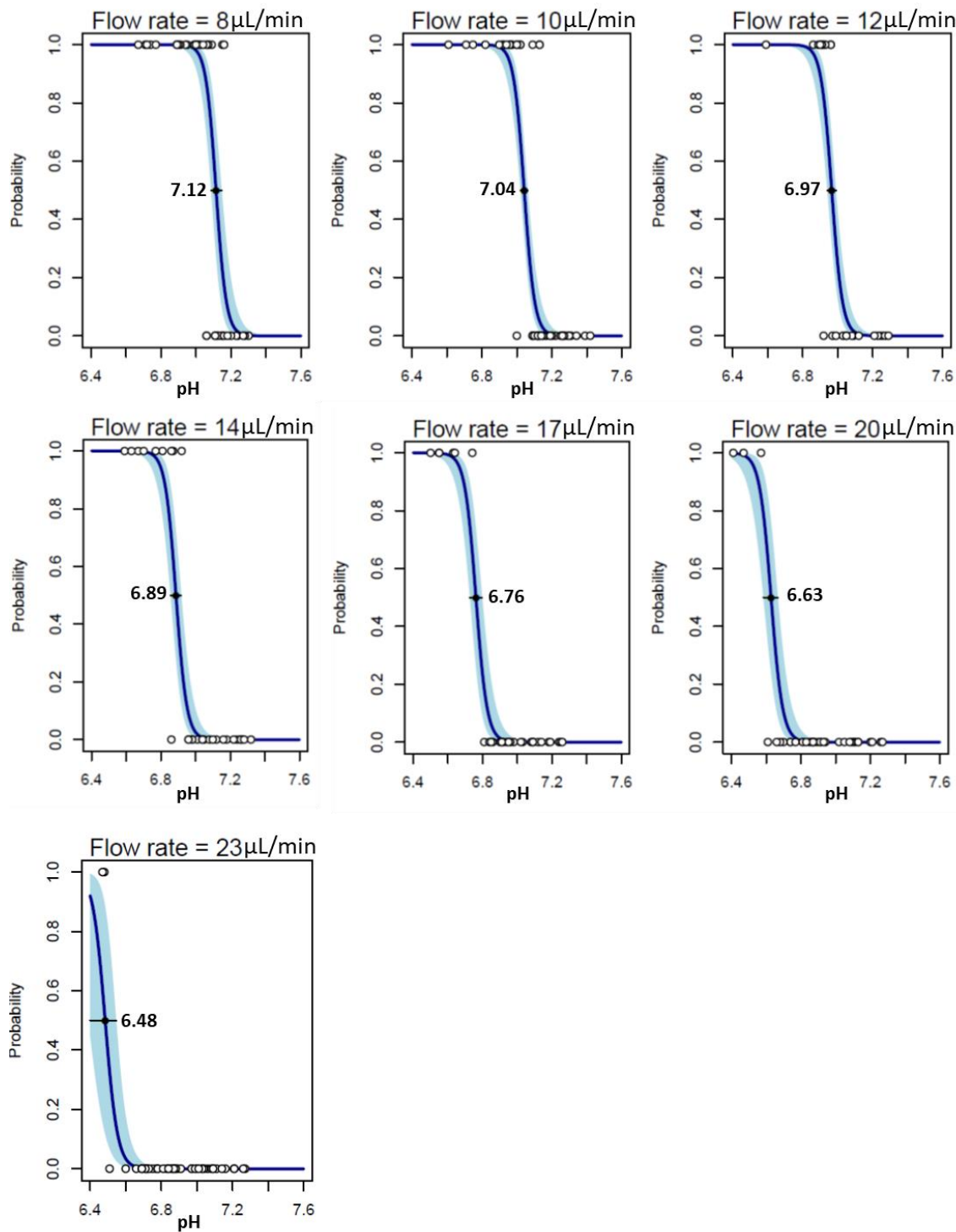
**Supplemental Figure S1.** SIFT device channel geometry.



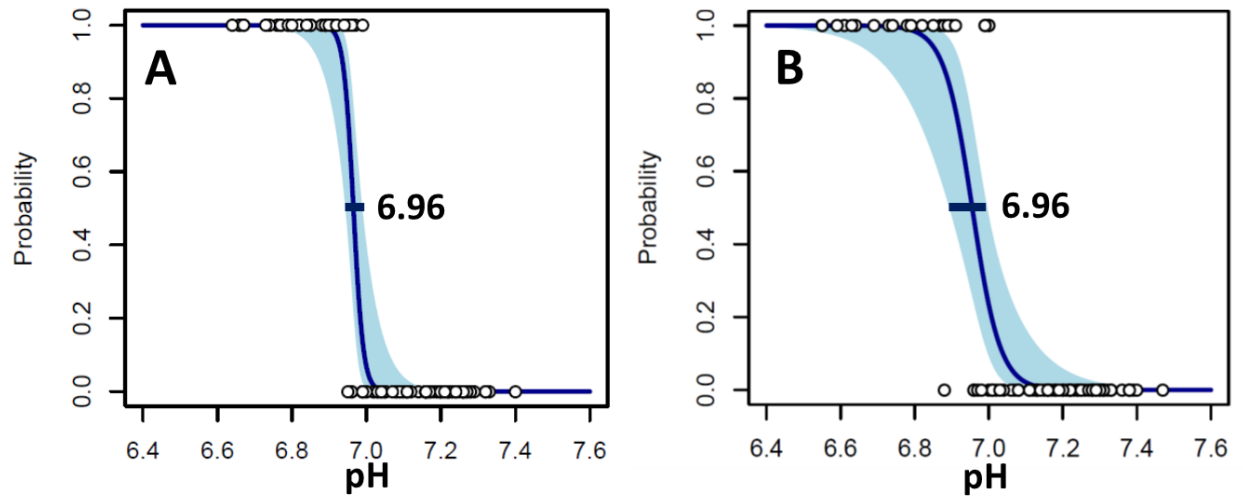
**Supplemental Figure S2.** Sorting rail dimensions and position. Exact position of rail is approximate as layers are positioned by eye.



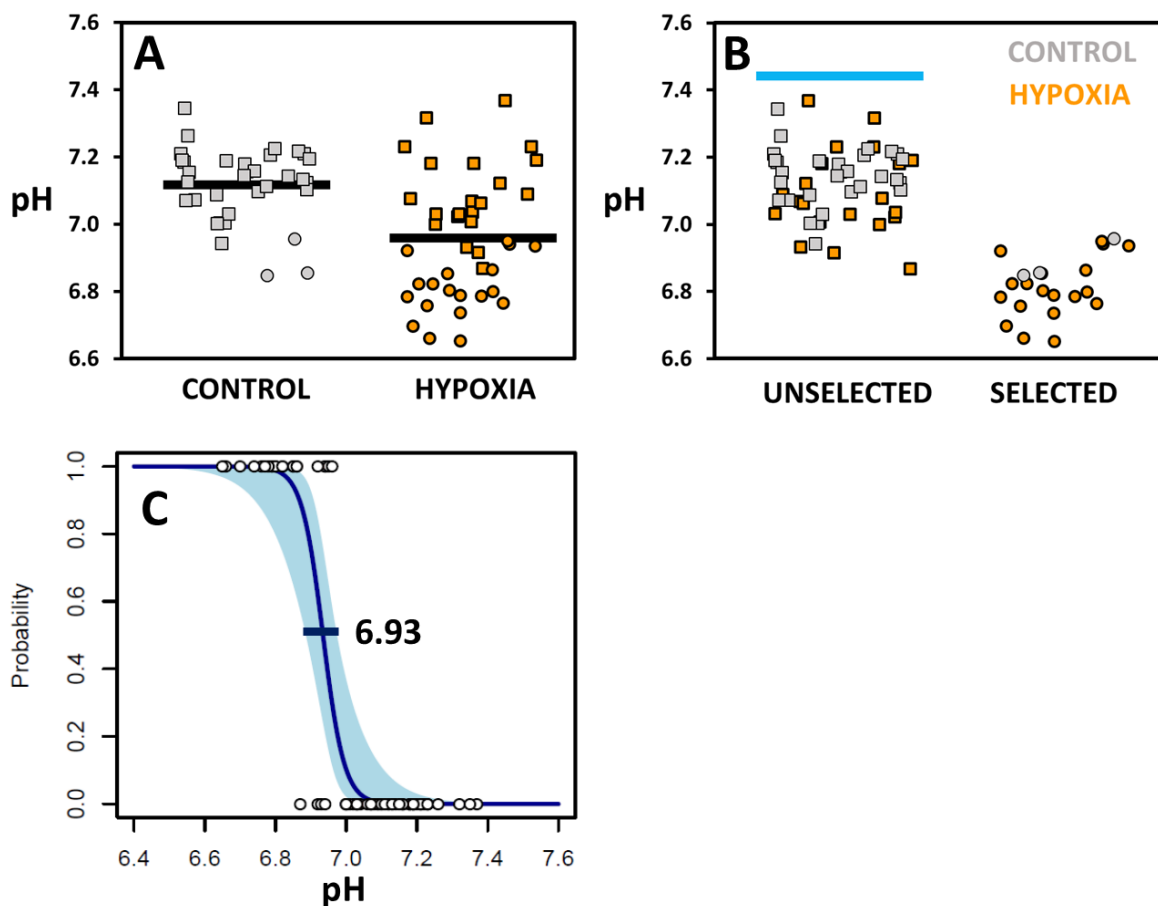
**Supplemental Figure S3.** Blue to violet fluorescence intensity ratio vs pH of droplets containing pyranine, a pH sensitive ratiometric fluorescent probe. Solutions of known pH were injected into the microfluidic device and droplets were analyzed for their normalized fluorescence intensity through excitation with blue (440 nm) and violet (395 nm) light. The calibration curve was obtained under the same conditions as cellular experiments. The slope of the calibration curve was found to be consistent from day to day, whereas the y-intersection was adjusted for each experiment using the known pH of empty droplets.



**Supplemental Figure S4.** A multiple logistic regression fit of binary selected/unselected data with pH at multiple flow rates. pH thresholds are indicated on graph and represent where there is equal probability that droplets are selected or unselected. The 95% confidence limit is indicated in light blue.



**Supplemental Figure S5.** A logistic regression fit of binary selected/unselected data with pH for A) hypoxia treated and control MDA-MB 231 cells and B) 2DG treated and control K562 cells. pH thresholds are indicated on graph and represent where there is equal probability that droplets are selected or unselected. The 95% confidence limit is indicated in light blue.



**Supplemental Figure S6:** pH of droplets containing either control (grey) or hypoxia treated (orange) K562 cells. Square markers annotate unselected, circular markers selected droplets. pH values were determined via a fluorescence intensity ratio. A) Droplets presented as control and hypoxia treated cell populations. Average pH values, indicated by a black line, are  $7.12 \pm 0.02$  ( $N = 34$ ) for control and  $6.96 \pm 0.03$  ( $N = 39$ ) for hypoxia cells. B) Droplets presented as unselected and selected populations. Blue line indicates the pH of empty droplets ( $\text{pH } 7.40 \pm 0.04$ ). The sorting of droplets leads to an enrichment of hypoxia treated cells from 54 % ( $N = 39$ ) before sorting to 86 % ( $N = 19$ ) of selected cells. C) The pH threshold of selection was determined by a logistic regression fit and was found to be  $6.93 \pm 0.05$  (threshold  $\pm$  95% confidence interval).



**Supplemental Table S1.** Typical flow parameters used in experiments. Channel geometry is provided below for reference. Negative flows below are opposite in direction to the main flow in the channel.

Inlets and Outlets	Flow Rates ( $\mu\text{L}/\text{min}$ )
Aqueous Inlet	0.1 - 0.5, most commonly 0.3
Oil Inlet	3 - 5, most commonly 3
QX100 Inlet	2 - 5
Oil Entrainment Inlet	8 - 20
Oil Outlet	- 2.7 to -4.7
Oil and Droplet Outlet	-0.25 to -1

