Electronic Supplementary Material (ESI) for Lab on a Chip.

High-selectivity cytology via lab-on-a-disc western blotting of individual cells

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SUPPLEMENTARY INFORMATION

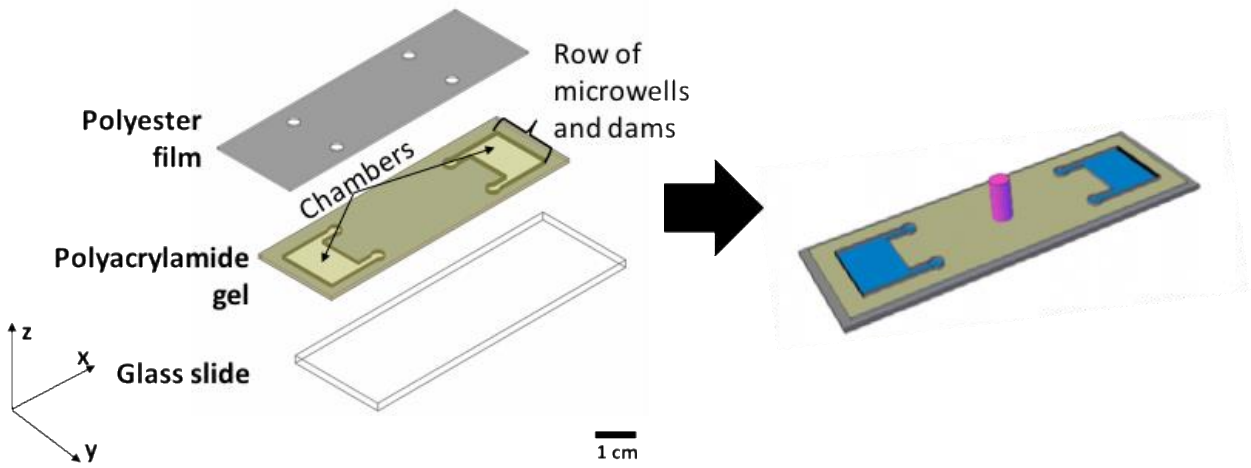

Fig. S1 Lab-on-a-disc device consists of a polyester film, polyacrylamide gel, and a microscope glass slide. The polyester lid encloses a cell suspension inside chambers during centrifugation. The microscope glass slide functions as a base support for the polyacrylamide gel and allows the imaging of cells and proteins during the device operation.

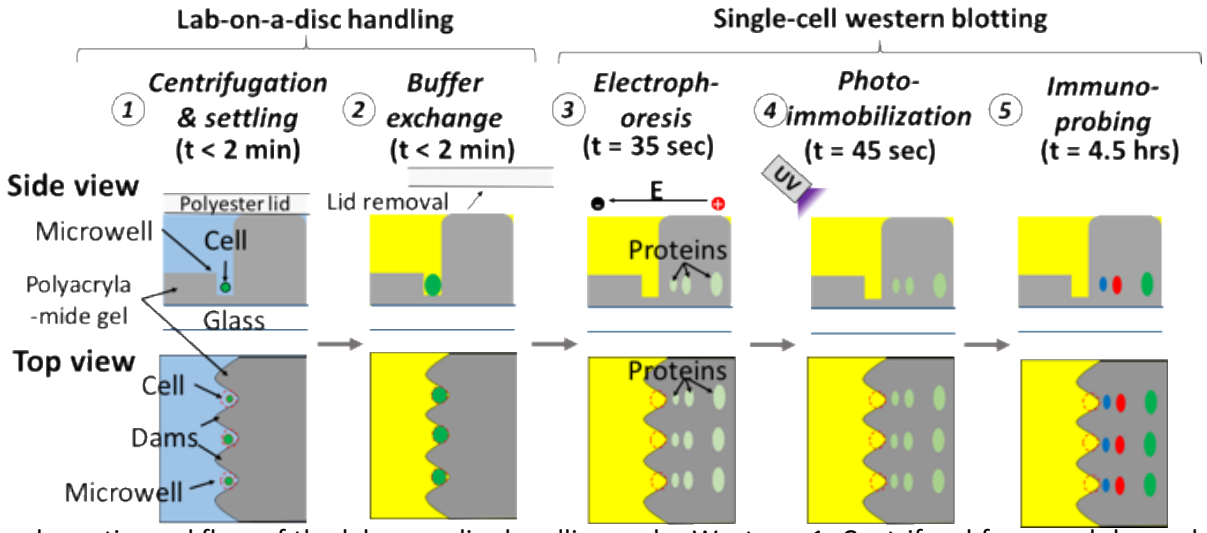

Fig. S2 A schematic workflow of the lab-on-a-disc handling and scWestern. 1: Centrifugal force and dams place single cells to microwells. Subsequently, cells settle in microwells by gravity. 2: After opening the polyester lid and cutting the device in half, a chemical lysis and electrophoretic buffer is poured on the device. Steps 3 – 5 entail scWestern. 3: An electric field (40 V/cm) is applied for protein separation. 4: UV is applied to activate benzophenone moieties, incorporated in the gel, for protein capture. 5: Fluorescent antibodies are introduced to probe for target proteins.

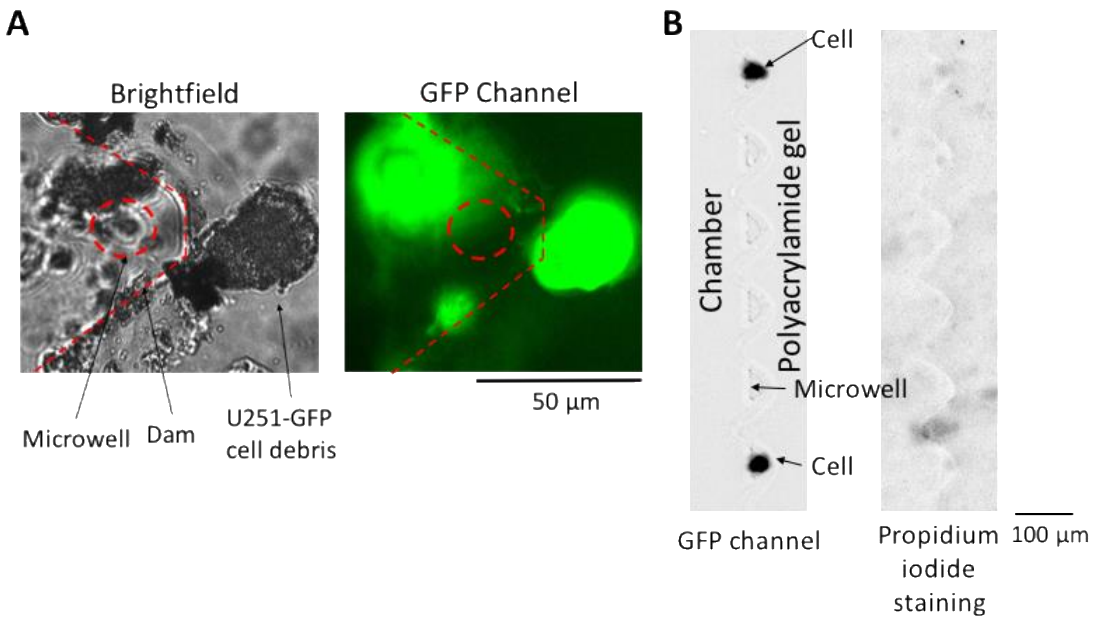

Fig. S3 Rotational speed affects U251-GFP cells in the lab-on-a-disc. (A) Centrifugation at 3000 rpm for 5 min results in a mechanical lysis of U251-GFP cells. Cell debris are detected near microwells in brightfield and GFP channels. (B) U251-GFP cells are stained with propidium iodide and centrifuged in the lab-on-a-device at 2000 rpm for 2 min. Absence of red fluorescence due to the propidium iodide inside the settled cells indicates that cells are viable after centrifugation.

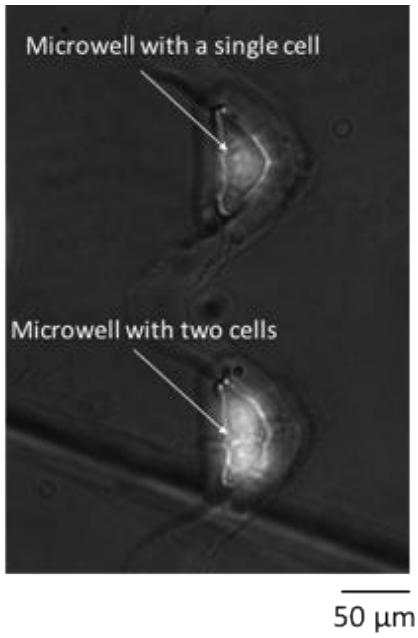

Fig. S4 The cell occupancy of each microwell is determined by combined brightfield and fluorescence inspection. Representative micrographs show 10x-objective fluorescence/brightfield images (100 ms exposure time) of the microwell region just prior to scWestern. (Top) Image of a single cell seated in a microwell and (bottom) image of multiple cells seated in a microwell. Using similar micrographs, we exclude scWestern endpoint protein readouts from microwells housing multiple cells.

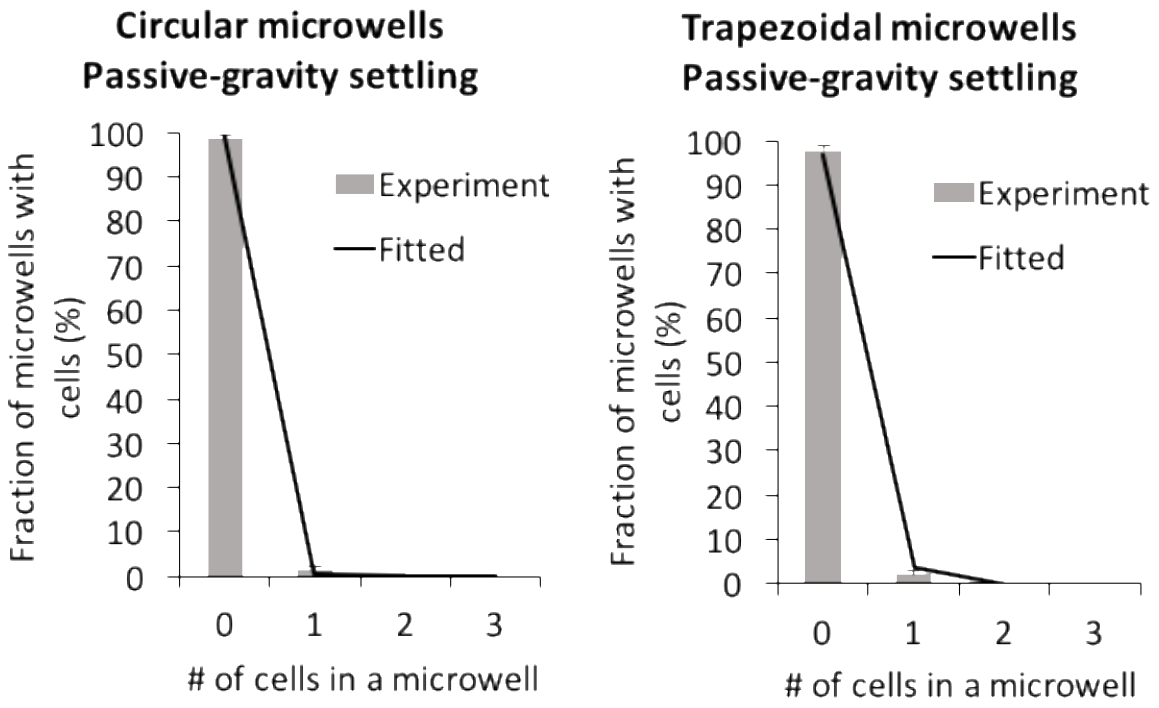

Fig. S5 Planar scWestern with passive-gravity settling contains < 4% of microwells filled with single cells and follows the Poisson's distribution with 100 cells (n = 4 slides for each case, λ = 0.002 for circular, 0.01 for trapezoidal).

$$\boldsymbol{U_{drift}} = \frac{dx}{dt} = \frac{x\omega^2(\rho_{cell} - \rho_{liq})d_{cell}^2}{18\mu}$$

$$\int_{r_1}^{r_2} dx \frac{18\mu}{x\omega^2(\rho_{cell} - \rho_{liq})d_{cell}^2} = \int_0^{t_1} dt$$

$$\boldsymbol{t_1 = \frac{18\mu}{\omega^2(\rho_{cell} - \rho_{liq})d_{cell}^2} ln(\frac{r_2}{r_1})}$$

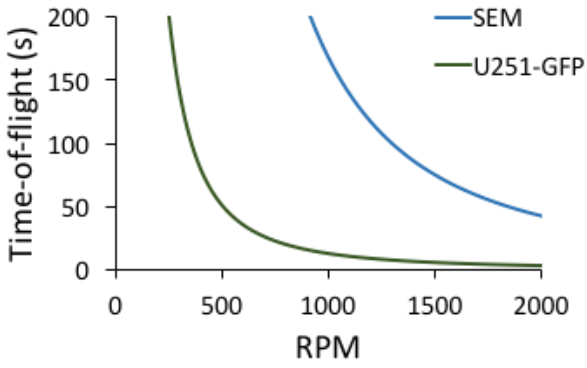

Fig. S6 Time-of-flight for size filtration of SEM cells from U251-GFP cells is calculated by integrating cell drift velocities with respect to radial distance and time. U_{drift} = cell drift velocity, μ = dynamic viscosity, ω = rotational velocity, r_1 = 0.023 m, r_2 = 0.033 m, ρ_{liq} = density of 1x PBS (0.995 g/mL), SEM cell diameter (d_{cell} = ~6 µm) and density (ρ_{cell} = 1.1 g/mL), U251-GFP cell diameter (d_{cell} = ~30 µm) and density (ρ_{cell} = 1.05 g/mL).

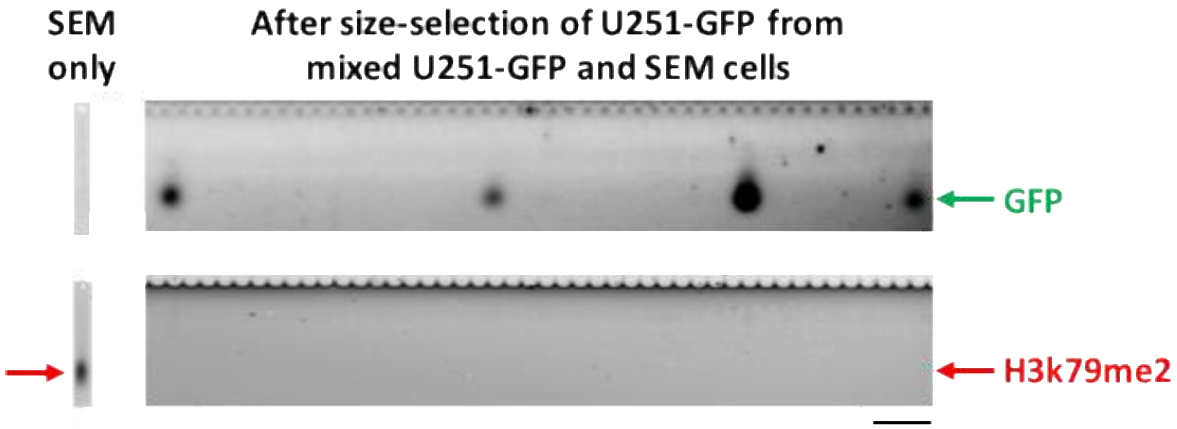

Fig. S7 scWesterns after sized-based separation of smaller SEM cells from larger U251-GFP cells. SEM only: micrograph from an scWestern of an SEM cell shows a positive peak for the 17kDa H3K79me2 protein, a leukemia-specific protein.[1] Micrograph of a scWestern blot for preferential cell seating of U251-GFP cells into microwells, using size-based separation of SEM cells from larger U251-GFP cells. scWestern reports the presence of U251-GFP cells only, as the H3K79me2 protein peak is absent. Scale bar is 500 µm.

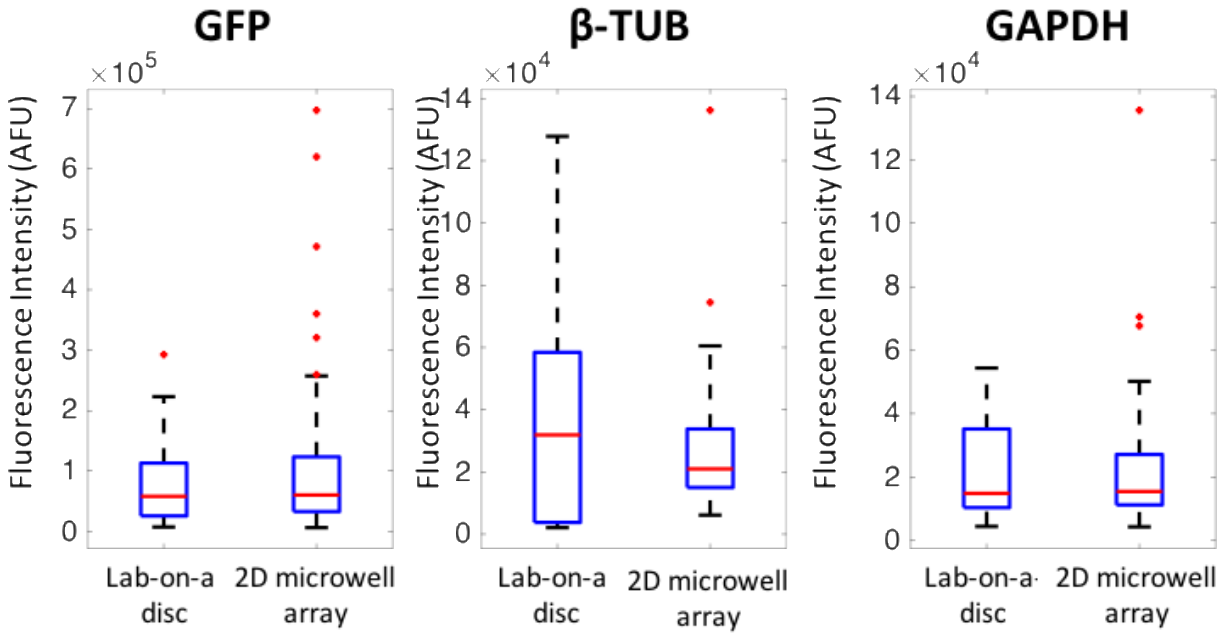

Fig. S8 GFP, β-TUB, and GAPDH peak intensities from U251-GFP cells are compared between the lab-on-a-disc and the planar (with gravity settling) scWesterns. The lab-on-a-disc and the planar scWesterns have no significant difference in fluorescence-intensity boxplots of GFP, β-TUB, and GAPDH. Blue box ends indicate 25th and 75th percentiles; median value is the red line at box middle; whiskers spread to 95% confidence limits; and red dots indicate outliers. (Mann–Whitney U-test, p-value of GFP = 0.54, p-value of β-TUB = 0.90, p-value of GAPDH = 0.65)

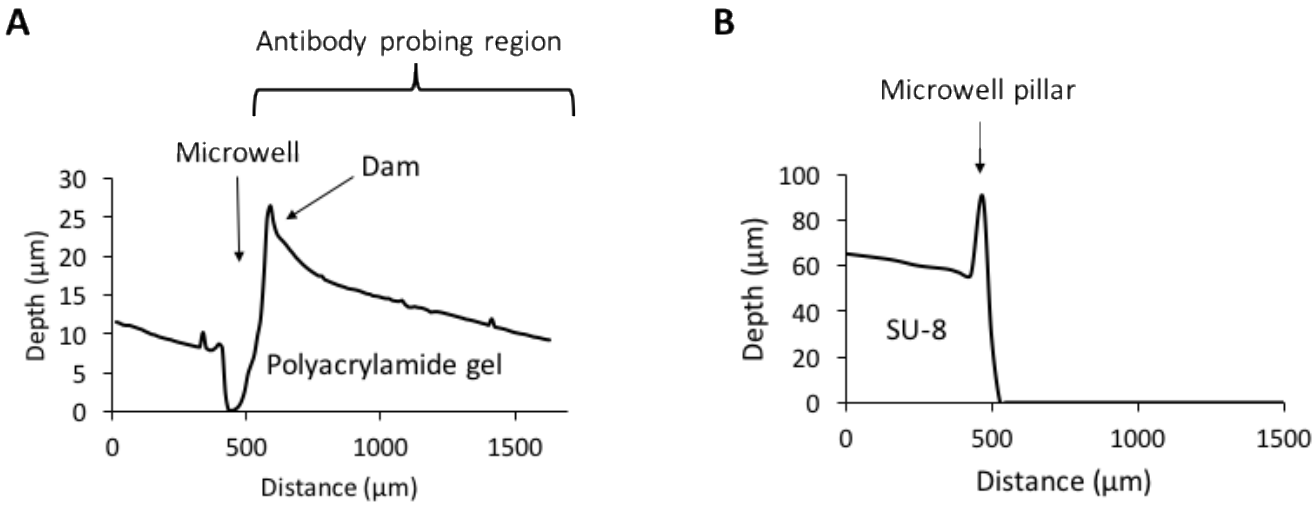

Fig. S9 Measurement of gel thickness for a lab-on-a-disc device indicates an uneven thickness (height) for a dried gel near the microwell array. (A) A surface profile of the dried polyacrylamide gel device reports a thicker gel near the microwell region, as compared to further along the separation axis. (B) A surface profile of the complement SU-8 mold suggests that the dehydration process used prior to antibody probing could be a source of the gel height non-uniformity.[2-4] Surfaces are profiled using Sloan Dektak 3030.

	GFP & GAPDH	GFP & β-TUB	GFP & STAT3	GAPDH & β-TUB	GAPDH & STAT3	β-TUB & STAT3
Mean	2.38	3.02	5.68	1.52	5.66	3.67
STDEV	1.42	1.63	3.33	0.49	3.28	2.05

Table S1 Separation resolutions between each protein peak from the lab-on-a-disc scWestern device.

Notes and references

1. K. M. Bernt, N. Zhu, A. U. Sinha, S. Vempati, J. Faber, A. V. Krivtsov, Z. Feng, N. Punt, A. Daigle, L. Bullinger, R. M. Pollock, V. M. Richon, A. L. Kung and S. A. Armstrong, *Cancer Cell*, 2011, **20**, 66-78.
2. K. Kudo, J. Ishida, G. Syuu, Y. Sekine and T. Ikeda-Fukazawa, *J Chem Phys*, 2014, **140**, 044909.
3. Y. Sekine and T. Ikeda-Fukazawa, *J Chem Phys*, 2009, **130**, 034501.
4. J. Vlassakis and A. E. Herr, *Anal Chem*, 2015, **87**, 11030-11038.