Electronic Supplementary Material (ESI) for Lab on a Chip

Supplemental Figure 1.
Maintenance of cell integrity permitting collection for re-analysis and capacity to measure retardation in cell mixtures. (A) Non Destructive Analysis of the Cells. P3 cells were analyzed for flow velocity and specific flow retardation using buffer containing 1.25% glycerol, a total flow rate of 30 μl/min in a 50 μm chamber height device. ΔH values over 500 millisecond intervals were measured while traversing coated patches. Cells were collected from the outflow tube and injected into the device for a second analysis, as shown in the second graph. (B) One to one mixtures of P3 and MDA-MB-231 cells were analyzed for flow velocity and specific flow retardation using buffer containing 1.25% glycerol, a total flow rate of 10 μl/min in a 25 μm chamber height device.

Supplemental Figure 2.
Comparison of 50 msec velocities at the single cell level. Horizontal axis is ΔH. Vertical axis is number of 50 msec intervals in each bin for a single cell. Data demonstrate distribution of all ΔH values measured for 50 msec intervals for 10 individual P3 and 10 MDA-MB-231 cells as they each sequentially traversed a BSA- and an IL13Rα2 antibody-coated patch at shear rate and force conditions described in figure 4. All P3 cells assessed individually had a trailing population of ΔH measured values over IL13Rα2 antibody-coated patches that were significantly slower than the rest of the distributed values on the same patch, than all of the values on the BSA-coated patches and slower than all of the values on both patches for MDA-MB-231 cells. These data convincingly supported the rationale for the use of the lowest ΔH value for comparing flow rate characteristics of two cell populations for determination of the presence of a surface antigen recognized by an immobilized ligand.

Supplemental Figure 3.
Flow retardation determined by differences in ΔH values measured at 50 msec intervals is also subject to shear rates and force. The data depicts the distribution of mean ΔH values measured at 50 millisecond intervals under the worst shear rate and force conditions, previously calibrated, with the chamber height at 75 μm, the flow rate at 15 μL/min and in buffer without glycerol. Specificity of flow retardation seen in P3 cells remains intact but sensitivity is greatly diminished in identifying P2 and P3 cells.

Supplemental Fig. 4.
Measurements of individual cell velocities collected over 50 millisecond intervals as each cell is observed traversing a BSA-coated and a pooled goat IgG-coated control patch under favorable shear rates at 25 micron height, 1.25% glycerol and 5 microliters/min. (A) All of the ΔH values measured from each cell at 50 millisecond intervals over each patch plotted against observation number in all 5 cell lines. (B) Minimum ΔH values of the same cells graphed in A. as they are measured at 50 millisecond intervals over the two patches. Data demonstrate no differences in passage velocities between the two patches.

Supplemental Fig. 5.

Blocking of cell retardation with pooled IgG or anti-IL13Rα2 for P1 and P3 cells. Prior to analysis, cells were incubated with 250 ng/ml antibody or homotypic IgG for one hour. Horizontal axis depicts minimum delta H values and the vertical axis depicts the number of cells.

Supplemental Table 1. Mean delta H values on IL13Rα2 antibody-coated patches under unfavorable shear rate conditions depicted in Supplemental Figure 3.

Cell Line	Device Coating	Cell No.	Avg. ΔH	St. Dev.	% CV[1]	% Decrease[2]	P value
MCF-7	BSA	108	5.18	0.31	6%	0.3%	0.40493
	IL13Rα2 Ab	97	5.17	0.40	8%		
MDA-MB-231	BSA	103	5.24	0.31	6%	-2.8%	< 0.001
	IL13Rα2 Ab	104	5.39	0.22	4%		
LM2 P1	BSA	98	5.32	0.28	5%	1.4%	< 0.001
	IL13Rα2 Ab	103	5.25	0.29	6%		
LM2 P2	BSA	104	5.21	0.33	6%	3.4%	< 0.001
	IL13Rα2 Ab	98	5.03	0.43	9%		
LM2 P3	BSA	94	5.17	0.35	7%	10.4%	< 0.001
	IL13Rα2 Ab	99	4.63	0.35	8%		

[1]Coefficient of variation (St. Dev./Avg. ΔH x 100%)
[2]Change in Avg. ΔH between IL13Rα2 Ab-coated and BSA-coated patch

Supplemental Table 2. Mean cell velocities on IgG- and BSA-coated patches depicted in Supplemental Figure 4

Cell Line	Device Coating	Cell No.	Avg. ΔH	St. Dev.	% CV[1]	% Decrease[2]	P value
MCF-7	BSA	72	1.642	0.068	4%	-1.0%	0.09137
	Goat IgG	69	1.658	0.069	4%		
MDA-MB-231	BSA	58	1.644	0.123	7%	0.2%	0.12749
	Goat IgG	56	1.640	0.068	4%		
LM2 P1	BSA	66	1.636	0.086	5%	-0.4%	0.40329
	Goat IgG	60	1.642	0.099	6%		
LM2 P2	BSA	60	1.636	0.124	8%	-1.5%	< 0.001
	Goat IgG	55	1.661	0.075	5%		
LM2 P3	BSA	65	1.647	0.066	4%	0.2%	< 0.001
	Goat IgG	68	1.643	0.067	4%		

[1]Coefficient of variation (St. Dev./Avg. ΔH x 100%)
[2]Change in Avg. ΔH between IL13Rα2 Ab-coated and BSA-coated patch