Supplementary Information

Single-Cell Mechanical Characteristics Analyzed by Multi-Constriction Microfluidic Channels

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1. Fabrication

The microchannel was molded with two layer SU-8 for PDMS soft-lithography, which requires a good strength and adhesion. Therefore, we used SU-8 3005 (MicroChem, Newton, MA) on a clean and dehydrated silicon prime wafer. Compared to SU-8 2000 series, SU-8 3000 series have an increased adhesion strength of 69 mPa, where SU-8 2000 series have only 38 mPa. The fabrication procedure was illustrated as the "microfluidic device fabrication" in Figure 2. The first layer SU-8 3005 was spin coated at 2000 rpm with an acceleration of 400 r/s for 30 s. This SU-8 layer was around 8 μm in thickness. Then, the wafer was soft baked at 65°C for 10 min and followed by 95°C for 35 min. After the wafer with uncured SU-8 was cooled down to room temperature, the wafer was moved to a mask aligner (Karl Suss MA-6, SUSS MicroTech, Inc., Corona, CA) and covered with a negative photo mask with patterned cavity and channel structures. The exposure time was set to 40.7 s at 8.6 mW/cm^2, which provided a total dose of 350 mJ/cm^2 of i-line (365 nm) UV on the wafer. After the first UV exposure, the wafer was transferred to a hot plate for post exposure bake at 65°C for 10 min and followed by 95°C for 30 min. After the wafer cooled down to room temperature after the post bake, the wafer was immersed in SU-8 developer (MicroChem, Newton, MA) for 5 min to remove the uncured SU-8. The wafer with the first layer SU-8 pattern with the constriction channel structures was cleaned by isopropanol and DI water, then dried with a nitrogen gun.

The second layer SU-8 was spin coated on the wafer with SU-8 3025 (MicroChem, Newton, MA) at 3000 rpm with an acceleration of 500 r/s for 35 s. This SU-8 layer was around 25 μm in thickness. The wafer was then soft baked at 65°C for 15 min and followed by 95°C for 60 min. After the wafer with uncured SU-8 was cooled down to room temperature, the wafer was moved to a mask aligner and covered with a second negative photo mask with the patterned cavity structure, which was aligned to the first layer of SU-8 channels. The exposure time was set to 58.1 s at 8.6 mW/cm^2, which provided a total dose of 500 mJ/cm^2 on the wafer. After the first UV exposure, the wafer was transferred to a hot plate for post exposure bake at 65°C for 10 min and followed by 95°C for 30 min. After the wafer cooled down to room temperature after post bake, the wafer was immersed in SU-8 developer for 10 min with gentle agitation to remove the uncured SU-8. Then the wafer with the second layer SU-8 pattern was cleaned by isopropanol and DI water, then dried with a nitrogen gun.

Tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane (TFOCS, Fisher Scientific) was coated on the surface of the molds for the easy release of PDMS. 0.3 mL of TFOCS was dropped on the surface of a petri-dish, with the mold placed next to the droplets. Then, the petri-dish was moved into a vacuum chamber for 30 min. The TFOCS fully evaporated and formed a Teflon-like surface on the SU-8 mold. After the mold was prepared, standard PDMS replica molding was conducted to fabricate microchannel.

PDMS pre-polymer (SYLGARD® 184 silicone elastomer, Dow Corning, Midland, MI) and curing agent (SYLGARD® 184 silicone elastomer curing agent, Dow Corning, Midland, MI) mixture with a weight ratio of 10:1 was poured on the silicon with the SU-8 mold. The mixture was then placed in a vacuum container for 30 min to remove all the air bubbles. The degassed PDMS mixture was poured onto the mold and placed in a 65°C oven for 24 hours for the solidification of PDMS. The PDMS channels were then bonded to a glass slide after air plasma treatment using plasma cleaner (Harrick Plasma, model PDC-001, Ithaca, NY).

2. Cell video

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Before each experiment, we observe the morphology of the cells under microscope before trypsinization. After confirming that the cells are confluent and healthy, we used trypan blue to test the viability of the cells. For example, MCF-10A with passage number 16, with pH ~7.1, last passage date Oct. 5th, trypsinization date Oct. 10th, was used in experiment. MDA-MB-231 with passage number 34, pH ~7.0, last passage date Sept. 20th, trypsinization date Sept. 23rd, was used. The cells we used before experiment were health with 100% viability.

3. Statistic analysis

There is no significant difference between different batches of devices and cells. We repeated the experiment several times with 4 devices. The four blind-testing samples were from 4 new devices.

We used t-test to find the proper correlation between the initial velocity of MDA-MB-231 cells (cancer cells, represented as "CA") and MCF-10A cells (normal cells, represented as "NR"). Based on the t value of the two target samples, we can find how significant the two sets of data are correlated. The definition of t value is:

$$t_{CA\&NR} = \frac{\overline{V_{CA}} - \overline{V_{NR}}}{\sqrt{\frac{\sigma_{CA}^2}{n_{CA}} + \frac{\sigma_{NR}^2}{n_{NR}}}}$$

where $\overline{V_{CA}}$, σ_{CA}, and n_{CA} is the average value, standard deviation, and sample size of the cancer cells sample, respectively; and $\overline{V_{NR}}$, σ_{NR}, and n_{NR} is the average value, standard deviation, and sample size of the normal cells sample, respectively. We assume the initial entry velocity

$$\overline{V_{CA}} = k \cdot \overline{V_{NR}}$$

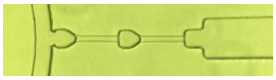
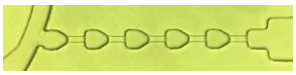
where k represent how much times the velocity of cancer cells is compared to normal cells.

$$t_k = \frac{\overline{V_{CA}} - k \cdot \overline{V_{NR}}}{\sqrt{\frac{\sigma_{CA}^2}{n_{CA}} + \frac{k^2 \cdot \sigma_{NR}^2}{n_{NR}}}}$$

Using α = 0.05, we can find the $t_k = 1.647$; therefore, k=1.92. This means that the probability of the initial velocity of cancer cells is 1.92 times to normal cells is larger than 95%. If we let $k = 2$, which means $\overline{V_{CA}} > 2 \cdot \overline{V_{NR}}$, the possibility p = 79.87%. Similarly, if $\overline{V_{CA}} > 1.86 \cdot \overline{V_{NR}}$, the possibility p = 99%; if if $\overline{V_{CA}} > 1.79 \cdot \overline{V_{NR}}$, the possibility p = 99.9%.

4. Differentiate criteria

Table S-1. Differentiate ratio of the cancer cells (CA) and the normal cells (NR) in channel 1, 2, and 3 using different criteria.

Observed \ Predicted	CA	NR	Criteria	Microfluidic channel
CA	1.2%	2.7%	I	 Channel 1
NR	98.8%	97.3%		
CA	16.5%	5.4%	II	
NR	83.5%	94.6%		
CA	35.3%	14.9%	III	
NR	64.7%	85.1%		
CA	52.9%	13.5%	II & III	
NR	47.1%	86.5%		
CA	3.3%	0%	I	 Channel 2
NR	96.7%	100%		
CA	22.3%	6.1%	II	
NR	77.7%	93.9%		
CA	66.9%	18.2%	III	
NR	33.1%	81.8%		
CA	83.5%	11.1%	II & III	
NR	16.5%	88.9%		
CA	43.5%	0%	I	 Channel 3
NR	56.5%	100%		
CA	77.8%	5.7%	II	
NR	22.2%	94.3%		
CA	91.7%	17.1%	III	
NR	8.3%	82.9%		
CA	94.4%	5.7%	II & III	
NR	5.6%	94.3%		

5. Scattered plots

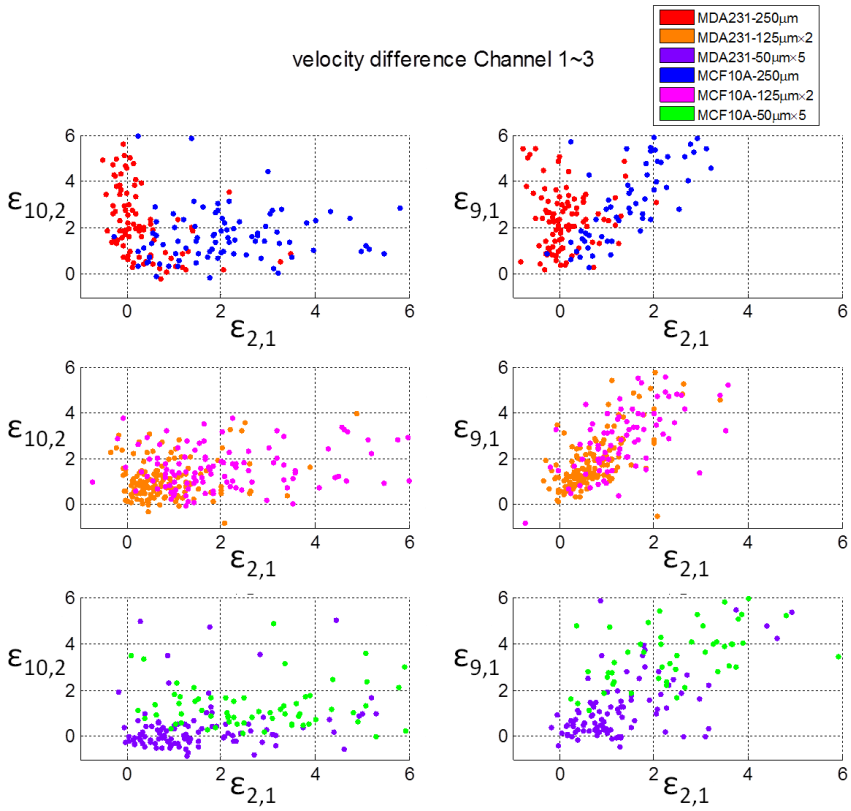


Figure S-1. Scattered plot of MDA-MB-231 and MCF-10A by comparing $\varepsilon_{10,2}$ to $\varepsilon_{2,1}$, and $\varepsilon_{9,1}$ to $\varepsilon_{2,1}$, in channel 1, channel 2, and channel 3, respectively.

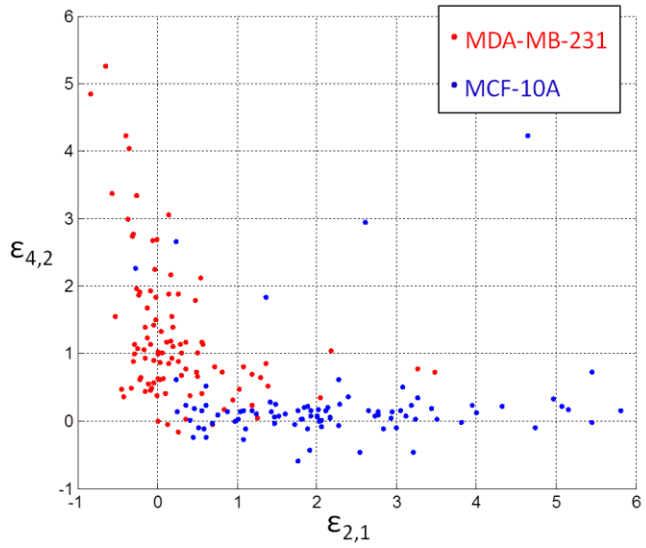


Figure S-2. Scattered plot of MDA-MB-231 and MCF-10A by comparing $\varepsilon_{4,2}$ to $\varepsilon_{2,1}$ in channel 1.

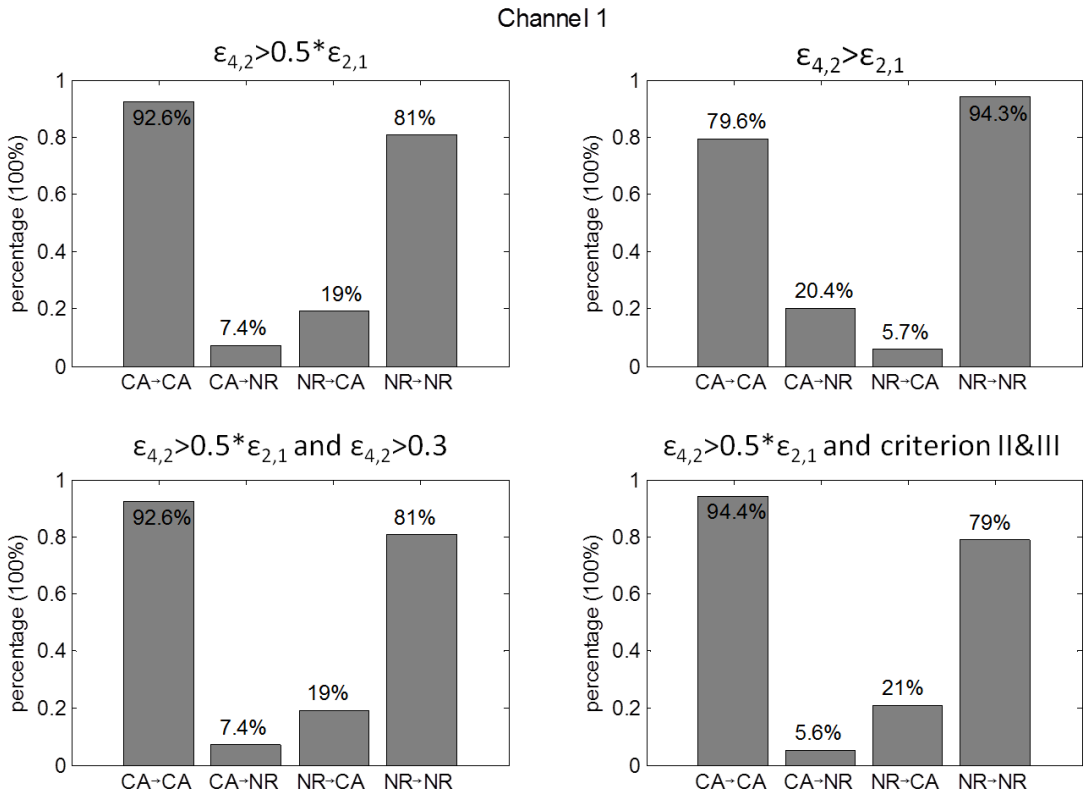


Figure S-3. Differentiate rate of cancer cells (CA) and normal cells (NR) in channel 1 using ε4,2 criterion.

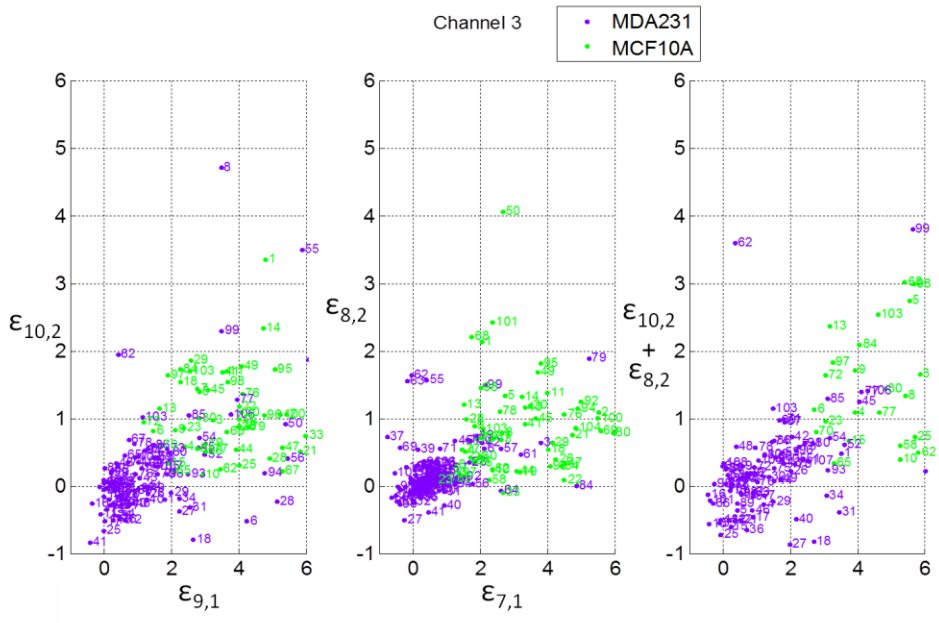


Figure S-4. Scattered plot of MDA-MB-231 and MCF-10A cells with sequence number for each dot.

6. ROC curves of differentiation criteria

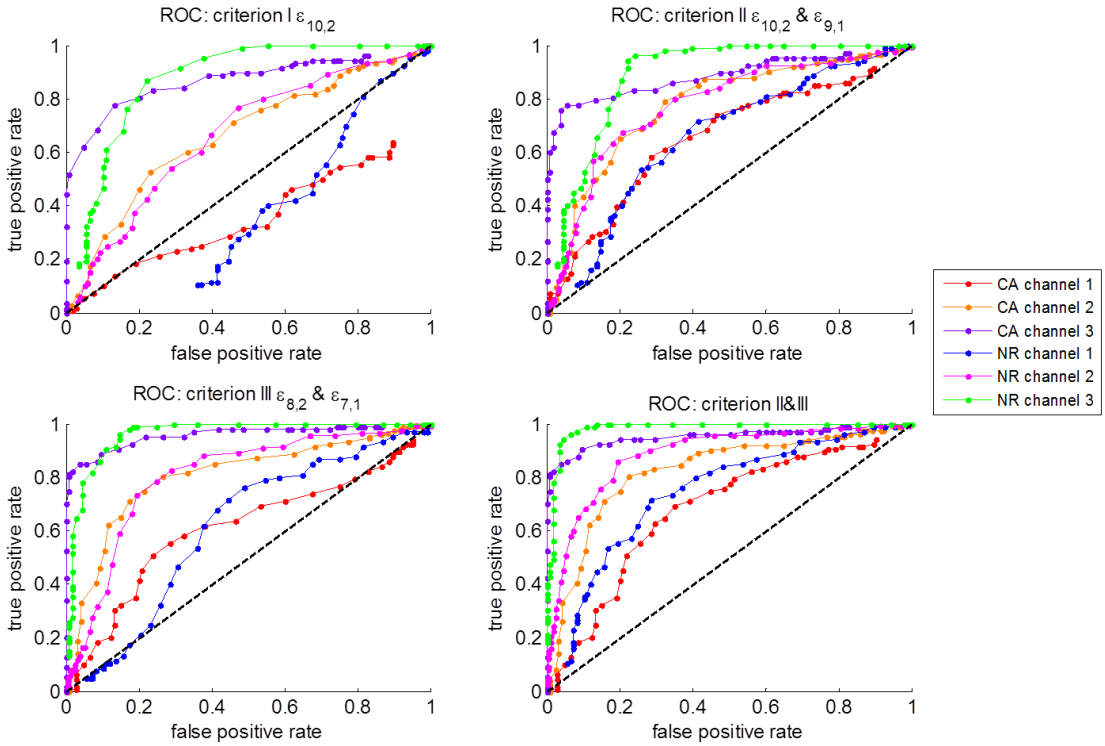


Figure S-5. ROC curve of criterion I, II, III, and II&III.

Table S-2. Sensitivities of criteria based on ROC curves.

		Criterion I	Criterion II	Criterion III	Criterion II&III
CA	Channel 1	–	0.74	0.61	0.69
	Channel 2	0.70	0.79	0.81	0.80
	Channel 3	0.78	0.78	0.89	0.90
NR	Channel 1	–	0.71	0.76	0.76
	Channel 2	0.76	0.80	0.83	0.86
	Channel 3	0.87	0.94	0.96	0.96