

## **Supplementary Information**

Supplementary Figure 1. Photograph of the microfluidic component. Scale bar, 10 mm.



**Supplementary Figure 2.** Position of HT-29 cells flowing in the microchannel. The inner walls of the microchannel are at 40  $\mu$ m and -40  $\mu$ m.



**Supplementary Figure 3.** Histograms of events in the SNR. Top, SNRs of EpCAM-channel images of HT-29 cells; middle, SNRs of PpIX-channel images of HT-1080 cells; bottom, SNRs of CD45-channel images of PBMCs. Arrows, peak position.

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**Supplementary Figure 4.** Images of all the events in the region of  $< 250 \,\mu\text{m}^2$  in the HT-29 population. Green, EpCAM-FITC; red, PpIX; blue, CD45-PE; scale bar, 20  $\mu$ m; arrow, flow direction (1 m/sec).



**Supplementary Figure 5.** Complete version of the scatter plots and histograms for the fluorescence intensity-based analysis of the mixture of HT-29 cells and PBMCs.



**Supplementary Figure 6.** Complete version of the scatter plots and histograms for the fluorescence intensity-based analysis of the mixture of HT-1080 cells and PBMCs.

Mixture of HT-29 cells and PBMCs



Mixture of HT-1080 cells and PBMCs



**Supplementary Figure 7.** Gating strategy for Fig. 6. First, events positive for either EpCAM-FITC or PpIX were gated. Then, events that are negative for CD45-PE or have lower CD45 coverage were gated. These gating regions are highlighted in red in the scatterplots.



**Supplementary Figure 8.** Scatter plots and gating strategy of the sampling test using 1,000 events extracted from events of the mixture of HT-29 cells and PBMCs. First, events positive for either EpCAM-FITC or PpIX were gated. Then, events that are negative for CD45-PE or have lower CD45 coverage were gated. These gating regions are highlighted in red in the scatter plots. Finally, the gated events were plotted in the circularity and integrated CD45-PE intensity with gating areas to classify the events into single cells, cancer-cell clusters, and clusters of a cancer cell(s) and a leukocyte(s).

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Images in gating area of single cells (n = 612 cells)

Images in gating area of cancer-cell clusters (n = 65 cells)

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Images in gating area of clusters of cancer cells and leukocytes (n = 12 cells)



**Supplementary Figure 9.** Image libraries of all events in each of the gating areas shown in Fig. S8. Green, EpCAM-FITC; red, PpIX; blue, CD45-PE; scale bar, 20 µm; arrow, flow direction (1 m/sec).



**Supplementary Figure 10.** Scatter plots and the gating strategy of the sampling test using 1,000 events extracted from events of the mixture of HT-1080 cells and PBMCs. First, events positive for either EpCAM-FITC or PpIX were gated. Then, events that are negative for CD45-PE or have lower CD45 coverage were gated. These gating regions are highlighted in red in the scatter plots. Finally, the gated events were plotted in the circularity and integrated CD45-PE intensity with gating areas to classify the events into single cells, cancer-cell clusters, and clusters of a cancer cell(s) and a leukocyte(s).

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Images in gating area of single cells (n = 525 cells)

Images in gating area of clusters of cancer cells and leukocytes (n = 22 cells)

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**Supplementary Figure 11.** Image libraries of all events in each of the gating areas shown in Fig. S10. Green, EpCAM-FITC; red, PpIX; blue, CD45-PE; scale bar, 20 µm; arrow, flow direction (1 m/sec).



**Supplementary Figure 12.** Scatter plots and the gating strategy for the rare cell detection experiments and clinical sample tests. The gating region is highlighted in red in the scatter plots.

Donor ID	Gender	Age	Preoperative/ postoperative	Postoperative histological type	Postoperative stage	Chemical therapy	Radiation therapy	Metastasis
Patient A	Female	37	Preoperative	Invasive ductal carcinoma	I	No	No	No
Patient B	Female	70	Preoperative	Invasive lobular carcinoma (left)/ Invasive ductal carcinoma (right)	Ш	No	No	No
Patient C	Female	66	Preoperative	Invasive ductal carcinoma	I	No	No	No
Patient D	Female	50	Preoperative	Invasive ductal carcinoma	I	No	No	No
Healthy donor A	Female	50	N/A	N/A	N/A	N/A	N/A	N/A
Healthy donor B	Female	26	N/A	N/A	N/A	N/A	N/A	N/A
Healthy donor C	Male	31	N/A	N/A	N/A	N/A	N/A	N/A
Healthy donor D	Male	27	N/A	N/A	N/A	N/A	N/A	N/A
Healthy donor E	Male	27	N/A	N/A	N/A	N/A	N/A	N/A
Healthy donor F	Male	28	N/A	N/A	N/A	N/A	N/A	N/A

Supplementary Table 1. Demographics and clinical characteristics of the healthy donors and patients involved in this study.

Supplementary Table 2. Details of blood specimens and collected PBMC fractions.

Blood specimen ID	Donor	Volume of drawn blood	Usage	Population of each constituent of the collected PBMC fraction (%)					
		(mL)		Lymphocyte	Monocyte	Neutrophil			
1	Patient A	10	Clinical test	85.0	9.90	5.05			
2	Patient B	9	Clinical test	78.7	15.5	5.83			
3	Patient C	10	Clinical test	82.9	8.82	8.42			
4	Patient D	10	Clinical test	84.5	9.75	5.78			
5	Healthy donor A	10	Clinical test	71.3	19.7	9.00			
6	Healthy donor B	10	Clinical test	86.0	10.2	3.80			
7	Healthy donor C	10	Clinical test	70.0	21.4	8.58			
8	Healthy donor D	10	Clinical test	85.5	8.86	5.61			
9	Healthy donor E	10	Spike test	80.9	12.9	6.29			
10	Healthy donor F	10	Rare cell test	83.3	11.7	5.02			

Donor	Imaging duration (sec)	Event rate (eps)	Population (events)
Patient A	2472	1068	2.64 × 10 <sup>6</sup>
Patient B	3016	942	$2.84 \times 10^{6}$
Patient C	809	616	4.99 × 10 <sup>5</sup>
Patient D	784	821	6.43 × 10 <sup>5</sup>
Healthy donor A	3210	1091	3.50 × 10 <sup>6</sup>
Healthy donor B	7213	1099	7.93 × 10 <sup>6</sup>
Healthy donor C	4094	1078	4.41 × 10 <sup>6</sup>
Healthy donor D	1590	1027	1.63 × 10 <sup>6</sup>

Supplementary Table 3. Details of the image acquisition for clinical samples.