

Supplementary Information for

Hybrid Double Spiral Microfluidic Chip for RBC-Lysis-free Enrichment of Rare Cells from Whole Blood

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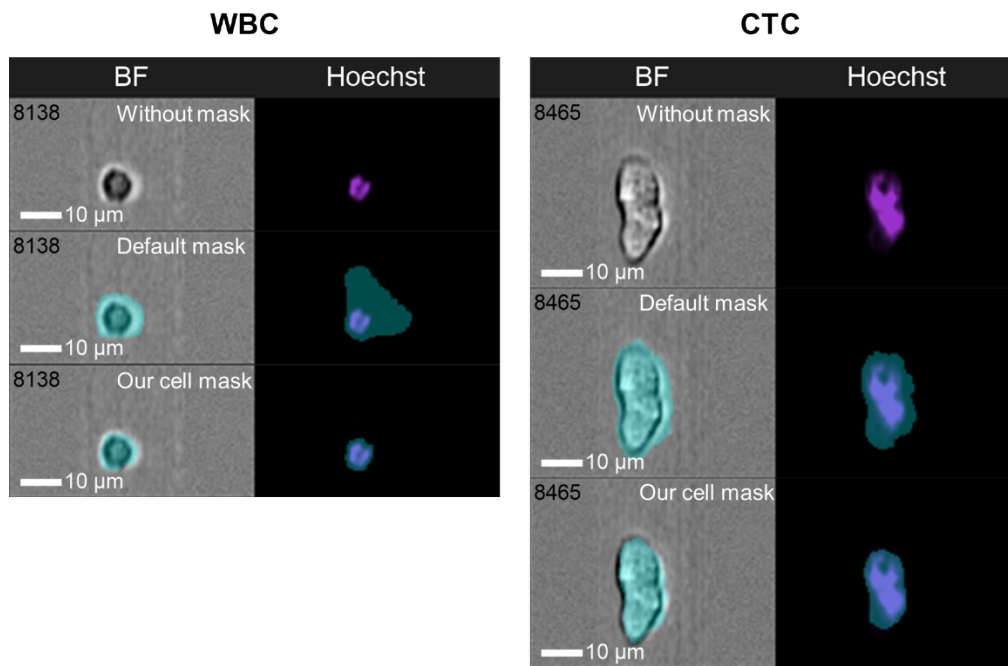
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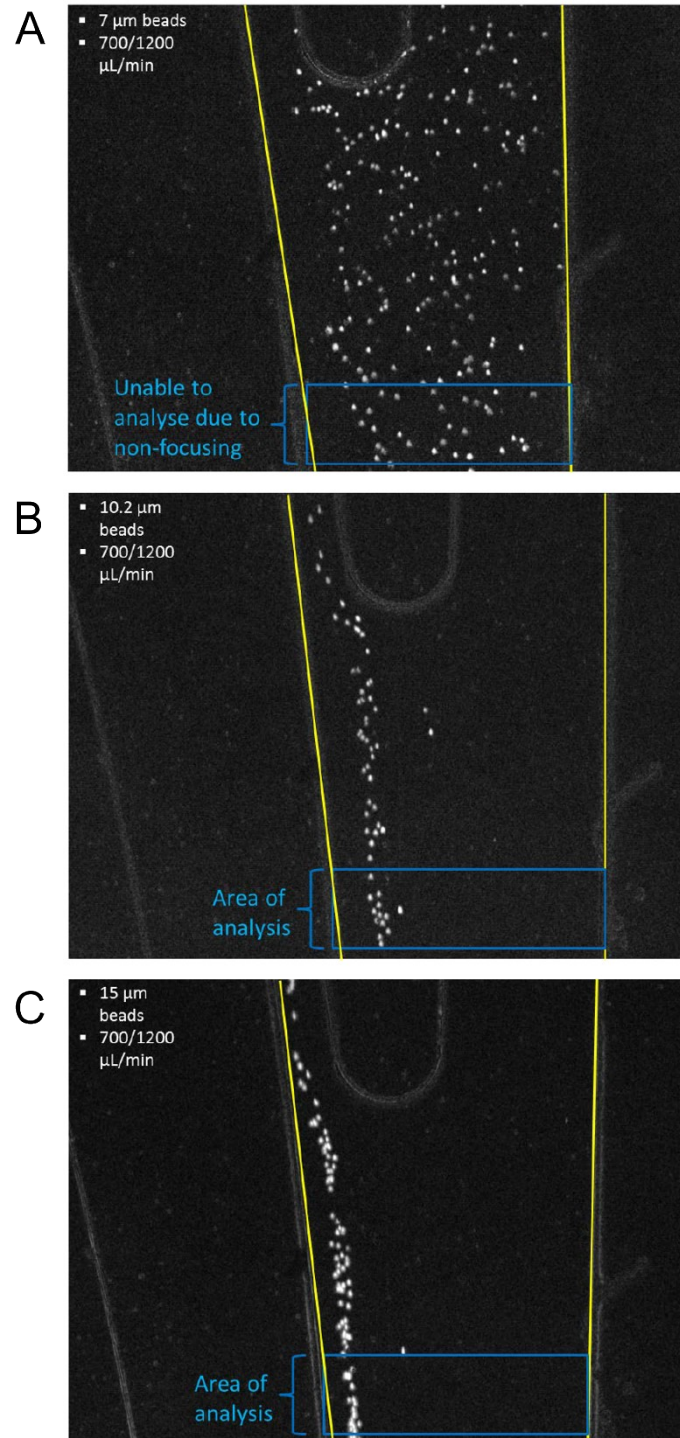
SI Figure 1. Cell size determination by using our cell mask. Images were taken from the breast cancer patient blood sample.

Default mask

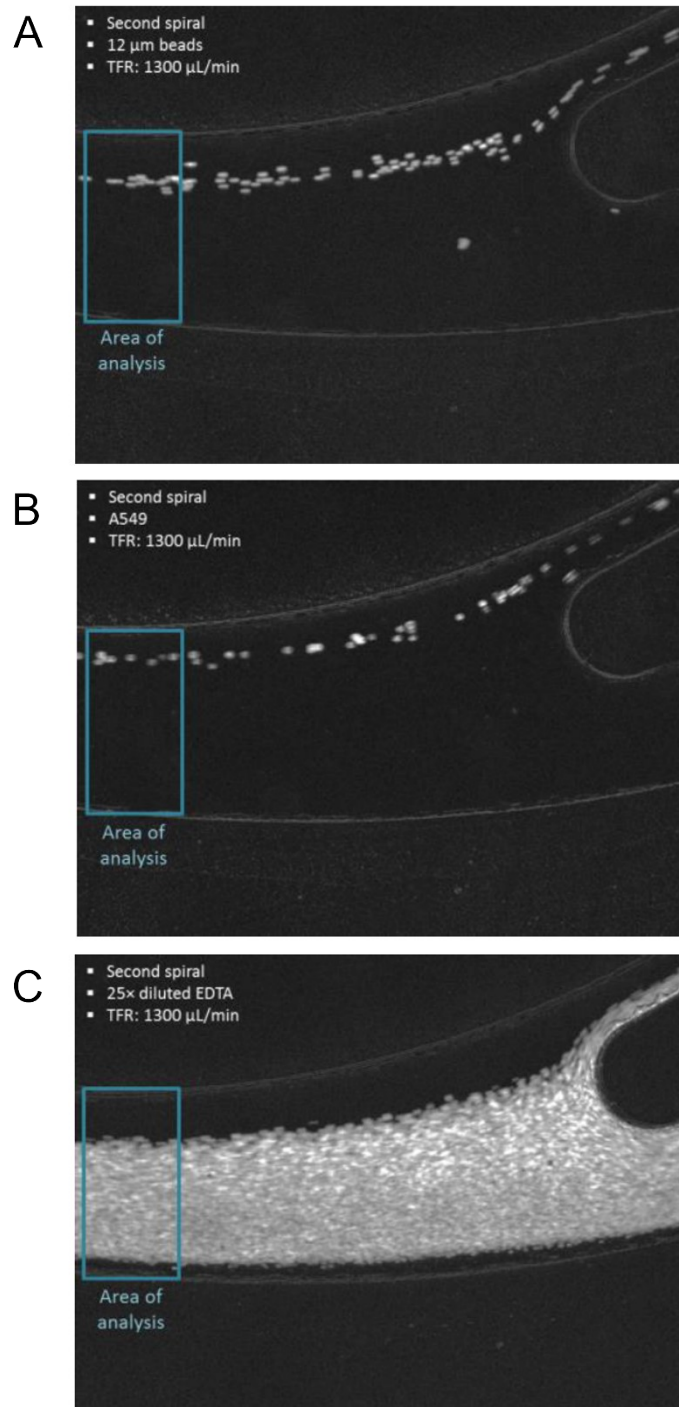
BF: M09, **Hoechst:** M07

Our cell mask

BF: Erode(M09, 3) Or Object(M07, Hoechst, Tight), **Hoechst:** Object(M07, Hoechst, Tight)



SI Figure 2. Size cutoff validation results of RBC removal chip (a half-Dean cycle chip) using microbeads of (A)7, (B)10.2, (C)15 μm . Videos of at least 30 frames near output bifurcation were taken for each under bright field. All the slices were processed by Z project the *Standard Deviation* using Image J software.



SI Figure 3. Size cutoff validation results of CTC/WBC separation chip (2nd spiral, a full-Dean cycle) of hybrid double spiral chip using (A)12 μm microbeads, (B)A549 cell line, (C)25X diluted EDTA blood. Measurement and analysis were performed with the same method as SI Figure 2.

SI Table 1. Conditions of cell staining and imaging flow cytometry detection

Cancer	Marker	Staining		Imaging flow cytometry detection(*)		
		Fix/Perm	Dye	Channel	Feature	Posi/Nega gating cutoff
Breast	Bright field (BF)	2% PFA/ 70% Methanol	-	Ch09	-	-
	Nucleus		Hoechst33142	Ch07	Intensity_MC_Ch07	10000
	Cytokeratin		Anti-CK- eFluor570	Ch03	Intensity_MC_Ch03	50000
	CD45/16/34		Anti-CD45/16/34- BV605	Ch10	Intensity_MC_Ch10	10000
Prostate	Bright field (BF)	2% PFA/ 0.5% Saponin	-	Ch09	-	-
	Nucleus		Hoechst33142	Ch07	Intensity_MC_Ch07	5000
	Cytokeratin		Anti-CK-FITC	Ch02	Intensity_MC_Ch02	10000
	CD45/16/34		Anti-CD45/16/34- PE-Cy7	Ch06	Intensity_MC_Ch06	25000

(*) Conditions of laser power intensity

Breast 405nm:40mW, 488nm:200mW, 561nm:200mW, 642nm:150mW

Prostate 405nm:40mW, 488nm:200mW, 561nm:off, 642nm:150mW

SI Table 2. A549 recovery rate [%] of RBC removal chip separation

	Donor 3	Donor 4	Donor 5
Run1	100.5	92.2	92.5
Run2	97.4	82.9	90.5
Run3	108.7	93.6	112.8
AVG	102.2	89.6	98.6
SD	5.8	5.8	12.4

SI Table 3. Cell numbers in CTC recovery solution of hybrid double spiral chip separation

Donor	Run	Cell counts in CTC recovery solution					Relative cell count ratio of each cell type			A549 purity% in WBC(*)
		RBC [cells]	WBC [cells]	A549 [cells]			RBC %	WBC %	A549 %	
				Spike	Recovery					
6	1	8.2.E+06	1.1.E+06	232	175	75.4%	88.4%	11.6%	0.0019%	0.02%
	2	1.7.E+06	3.3.E+05	143	116	81.1%	84.2%	15.8%	0.0056%	0.04%
	3	5.2.E+07	1.8.E+06	180	129	71.7%	96.7%	3.3%	0.0002%	0.01%
7	1	9.2.E+05	5.3.E+04	300	264	88.0%	94.5%	5.4%	0.0271%	0.50%
	2	5.1.E+05	9.8.E+04	315	290	92.1%	84.0%	16.0%	0.0475%	0.30%
	3	8.4.E+05	1.6.E+05	310	289	93.2%	84.3%	15.6%	0.0289%	0.18%
8	1	1.2.E+07	1.5.E+05	211	186	88.2%	98.7%	1.3%	0.0015%	0.12%
	2	5.1.E+05	1.2.E+05	242	182	75.2%	80.3%	19.7%	0.0289%	0.15%
	3	1.1.E+06	6.8.E+04	205	151	73.7%	94.0%	6.0%	0.0133%	0.22%
9	1	2.6.E+06	1.2.E+05	243	214	88.1%	95.6%	4.4%	0.0078%	0.18%
	2	9.1.E+06	8.4.E+04	265	224	84.5%	99.1%	0.9%	0.0024%	0.27%
	3	2.1.E+07	2.3.E+05	269	252	93.7%	98.9%	1.1%	0.0012%	0.11%
10	1	2.8.E+07	1.4.E+05	253	216	85.4%	99.5%	0.5%	0.0008%	0.16%
	2	1.9.E+07	1.1.E+05	244	226	92.6%	99.4%	0.6%	0.0012%	0.21%
	3	9.6.E+06	7.9.E+04	265	236	89.1%	99.2%	0.8%	0.0024%	0.30%

(*) A549 Purity% in WBC was defined as the percentage ratio of cell numbers of A549 cells to cell numbers of WBCs