Rapid, label-free CD4 testing using a smartphone compatible device

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Supplementary Materials

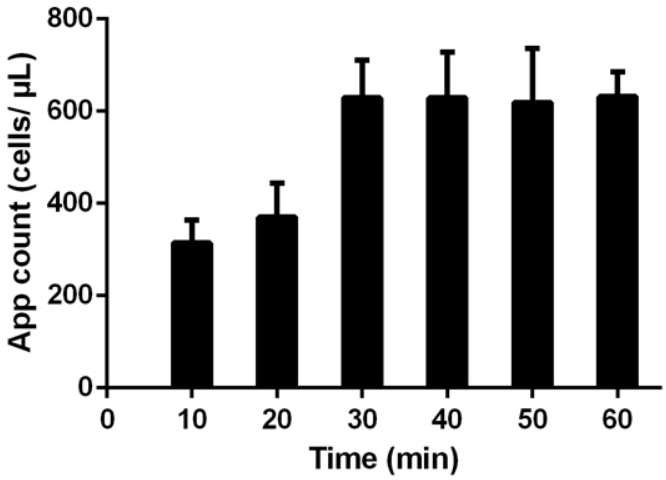

Figure S1. Optimization of on-chip cell capture incubation time. Whole blood samples were incubated on functionalized microchips between 10 and 60 minutes and the number of captured cells were counted at the end of the incubation times.

Cell capture efficiency and specificity evaluations

Captured cells were fixed with 4% (v/v) paraformaldehyde solution in PBS for 10 minutes. After fixation, cells were incubated with 0.2% (v/v) Triton X-100 for 5 minutes. Channels were washed with ice-cold PBS after every step. FITC anti-human CD4 antibody (357406, BioLegend) was added to the chip and incubated for 1 hour in a dark room at room temperature. After the incubation, the channel was washed and captured cells were stained with 0.2% (v/v) DAPI solution for 15 minutes in a dark room at room temperature. DAPI was obtained from Sigma-Aldrich (stock concentration: 5mg DAPI in 1 mL of deionized water). DAPI is a cell permeable fluorescent minor groove-binding probe for DNA which binds to the minor groove of double-stranded DNA, forming a stable complex.

A fluorescent microscope (Observer D1, Zeiss) was used for bright field and fluorescent imaging. Cells stained with blue represented DAPI, whereas cells in green represented CD4+ T

cells tagged with FITC. The ratio between the CD4+ T cells stained in green to other cells (green/blue) was measured to calculate the specificity.

Similarly, in evaluating cell capture efficiency, spiked PBS samples with known CD4 concentrations were loaded into microchips and were incubated for 30 minutes. We measured the number of cells on-chip before and after washing step. The cell capture efficiency was estimated to be 84%.

Cell capture efficiency was calculated by using the following formula:

$$Cell\ capture\ efficiency\ (\%) = \left(\frac{CD4\ cells\ cells\ after\ wash}{CD4\ cells\ before\ wash}\right) * 100$$

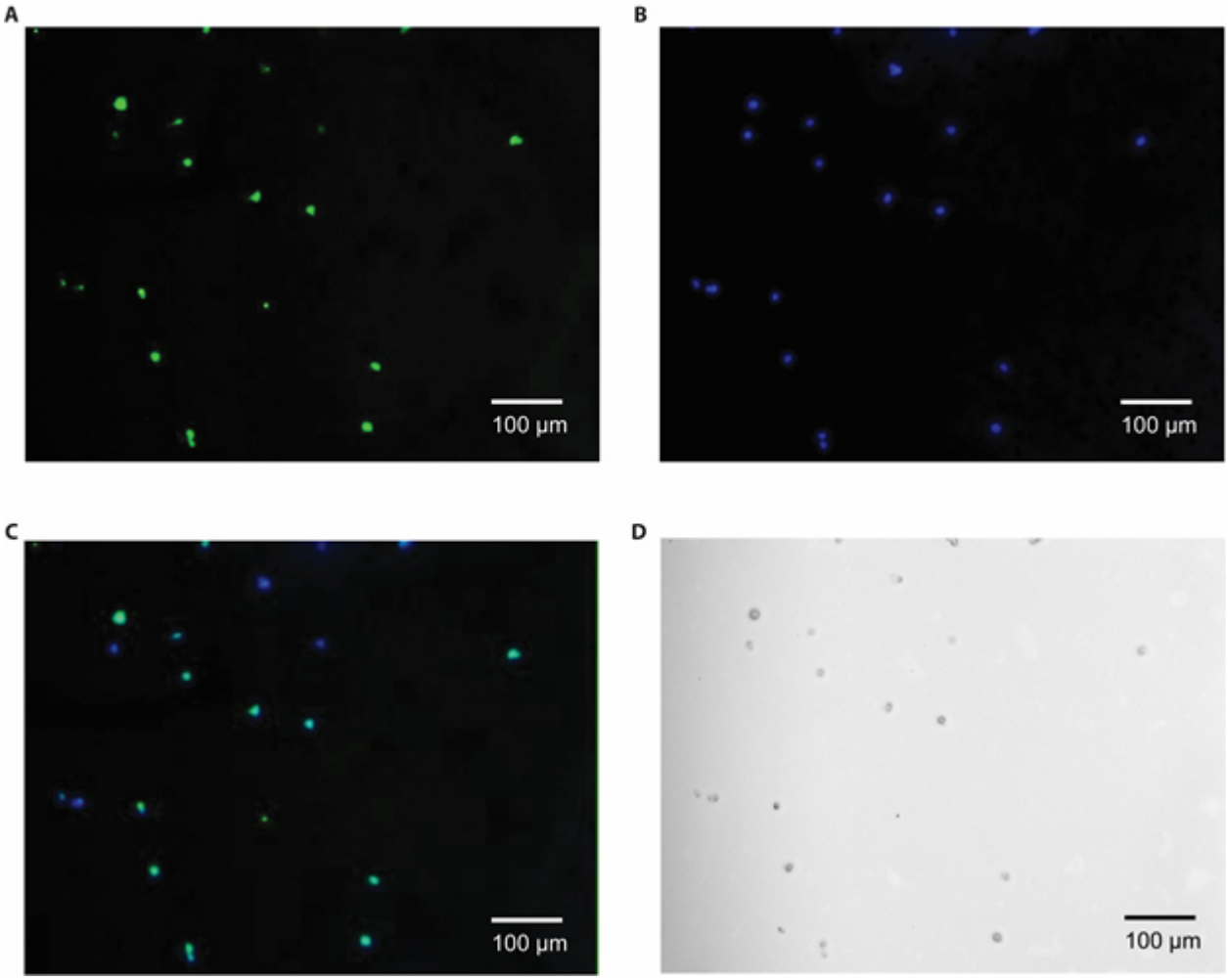

Figure S2. Specificity analysis using fluorescent images to identify the captured cells. (A) Fluorescent image of captured CD4 cells using whole blood and stained with FITC. The cells were also stained with (B) DAPI. (C) Image showing the overlay of the cells expressing both FITC and DAPI. (D) Bright field image of the cells captured from fresh whole blood. The images were all captured with a 10x objective.

Raw data – Isolated CD4 samples

Sample #	*Hemocytometer (cells/µL)*	*Smartphone application output (cells/µL)*			
		Replicate 1	Replicate 2	Replicate 3	Average
Sample 1	60	130	98	65	97.67
Sample 2	100	98	130	130	119.33
Sample 3	160	228	130	200	186.00
Sample 4	160	163	228	260	217.00
Sample 5	180	33	195	130	119.33
Sample 6	260	228	195	260	227.67
Sample 7	320	260	195	293	249.33
Sample 8	350	228	293	391	304.00
Sample 9	520	326	423	488	412.33
Sample 10	820	521	521	553	531.67
Sample 11	960	568	586	488	547.33
Sample 12	1000	879	749	911	846.33
Sample 13	1100	716	781	814	770.33
Sample 14	400	684	749	749	727.33
Sample 15	1640	1725	1888	1640	1751.00
Sample 16	680	553	163	326	347.33
Sample 17	800	423	1360	423	735.33
Sample 18	1040	977	553	619	716.33
Sample 19	680	423	358	488	423.00
Sample 20	220	130	163	163	152.00
Sample 21	260	130	130	98	119.33
Sample 22	240	293	98	98	163.00
Sample 23	160	163	130	98	130.33
Sample 24	60	98	65	33	65.33
Sample 25	30	0	0	0	0.00

Sample 26	20	0	0	0	0.00
Sample 27	0	0	0	0	0.00
Sample 28	420	390	391	326	369.00
Sample 29	460	423	326	326	358.33
Sample 30	300	298	228	293	273.00
Sample 31	180	228	163	228	206.33
Sample 32	160	163	130	228	173.67
Sample 33	800	621	684	651	652.00
Sample 34	660	781	684	521	662.00
Sample 35	740	619	684	716	673.00
Sample 36	760	781	721	716	739.33
Sample 37	580	521	521	586	542.67
Sample 38	980	1270	1042	1042	1118.00
Sample 39	820	1237	1270	1042	1183.00
Sample 40	800	1302	1074	944	1106.67
Sample 41	720	977	1074	846	965.67
Sample 42	600	553	521	553	542.33
Sample 43	1060	1074	916	1400	1130.00
Sample 44	1040	977	814	977	922.67
Sample 45	780	553	521	521	531.67
Sample 46	1000	911	1107	1270	1096.00
Sample 47	960	977	911	911	933.00
Sample 48	420	391	326	358	358.33
Sample 49	980	781	814	751	782.00
Sample 50	900	619	1270	621	836.67
Sample 51	800	684	749	1139	857.33
Sample 52	890	879	846	1107	944.00
Sample 53	1060	846	716	814	792.00

Sample 54	900	749	911	814	824.67
Sample 55	1200	1432	1335	1042	1269.67
Sample 56	120	98	130	163	130.33
Sample 57	60	65	65	163	97.67

Table S1. Raw sample data for all isolated CD4 samples tested without using functionalized microfluidic chips.

Raw data – Isolated CD4 samples

Sample #	***Hemocytometer (cells/µL)***	***Smartphone application (cells/µL)***			
		Replicate 1	Replicate 2	Replicate 3	Average
Sample 1	820	521	553	942	672.00
Sample 2	600	651	684	521	618.67
Sample 3	1000	1335	1204	1107	1215.33
Sample 4	960	456	488	423	455.67
Sample 5	980	553	619	586	586.00
Sample 6	1200	781	1042	1042	955.00
Sample 7	600	586	651	684	640.33
Sample 8	300	260	315	326	300.33
Sample 9	360	258	423	456	379.00
Sample 10	440	358	358	456	390.67
Sample 11	700	651	586	586	607.67
Sample 12	780	619	716	684	673.00
Sample 13	860	1009	840	1002	950.33
Sample 14	700	521	749	684	651.33
Sample 15	800	619	619	651	629.67
Sample 16	1200	1204	781	1042	1009.00
Sample 17	280	195	195	195	195.00

Sample 18	440	423	326	423	390.67
Sample 19	220	195	163	163	173.67
Sample 20	460	358	358	456	390.67
Sample 21	160	98	98	130	108.67
Sample 22	480	391	456	456	434.33
Sample 23	60	33	33	33	33.00
Sample 24	320	293	326	260	293.00
Sample 25	140	130	130	163	141.00
Sample 26	300	293	228	293	271.33
Sample 27	186.3	130	66	0	65.33
Sample 28	186.7	66	196	66	109.33
Sample 29	151	66	130	66	87.33
Sample 30	106.66	66	66	0	44.00

Table S2. Raw sample data for all isolated CD4 samples tested using functionalized microfluidic chips.

Raw data – Whole blood samples

Sample #	***Flow cytometer (cells/μL)***	***Smartphone application (cells/μL)***		
		Replicate 1	Replicate 2	Average
Sample 1	632	586	690	638
Sample 2	716	701	692	696.5
Sample 3	400	408	414	411
Sample 4	1114	1032	977	1004.5
Sample 5	991	813	1595	1204
Sample 6	1102	1237	1269	1253
Sample 7	581	456	651	553.5
Sample 8	512	391	456	423.5

Sample 9	1348	1302	1432	1367
Sample 10	221	195	326	260.5
Sample 11	598	456	521	488.5

Table S3. Raw data for whole blood samples tested using functionalized microfluidic chips.