Electronic Supplementary Material (ESI) for Lab on a Chip.
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Label–free, high-throughput holographic screening and enumeration of tumor cells in blood

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SUPPLEMENTARY INFORMATION

Table SM1: A comparison of minimum detectable spike in amount to non-spiked detection is presented. The concentration of spiked tumor cells is targeted to be10, 50, and 100 cells/mL of MCF7 and MDA-MB-231 each. The classification strategy corresponding to MCF7 and MDA-MB-231 has also implemented to non-spiked concentration (i.e. C = 0 cells/mL) to suggest a minimum detection limit. The total analyzed sample volume and number of cells (tumor cells + PBMCs) is 1 mL and 450,000 respectively.

Cell Line	Input Concentration (Cells/mL)	Spiked Cancer Cells in PBMCs from Donor 1				
		n_1	n_2	n_3	Mean	Standard deviation
MCF7	0	0	-	-	0	0
	10	17	4	18	13	8
	50	17	63	10	30	29
	100	67	73	113	84	25
MDA-MB231	0	6	-	-	0	0
	10	21	26	2	16.33	12.66
	50	59	8	63	43	31
	100	123	146	61	110	44

Table SM2: The detection of tumor cells in lysed blood of three different donors in non-spiked condition is presented. The classification strategy corresponding to MCF7 and MDA-MB-231 has been implemented to non-spiked concentration (i.e. C = 0 cells/mL) to suggest a minimum detection limit. The total analyzed sample volume and number of cells (tumor cells + PBMCs) is 1 mL and 450,000 respectively.

Cell Line	Input Concentration (Cells/mL)	Donor-1	Donor-2	Donor-3	Mean	Standard deviation
MCF7	0	0	3	1	1.33	1.5
MDA-MB231	0	6	3	0	3	3

Protocol adopted to obtain low concentration of spiked tumor cells

PBMCs were isolated from whole blood using ACK Lysing Buffer following manufacturer protocol. Suspensions of blood components were filtered with a 30 μm filter and diluted in PBS to a final concentration 0.45 million cells/mL. Adherent tumor cell lines, MDA-MB-231 and MCF-7, were cultured to 60-80% confluence, trypsinized using standard protocols, resuspended in PBS (1 million cells/ml), and serially diluted to 1000 cells/mL. For both MD-MBA-231 and MCF 7 samples, cancer cell suspensions were spiked in technical triplicate into PBMC suspensions (2 mL) at 20, 100 and 200 μL cancer cell suspension for target concentrations of 10, 50 and 100 cancer cells per mL. Finally, 1 mL of sample was analyzed corresponding to each data point. The counting of cells was performed by hemocytometer.