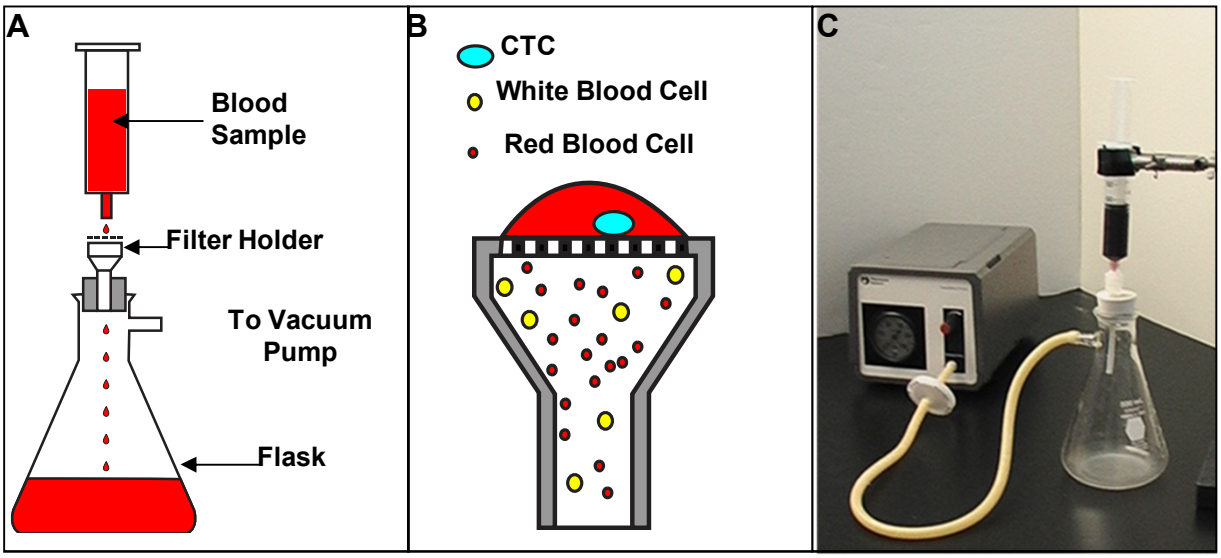
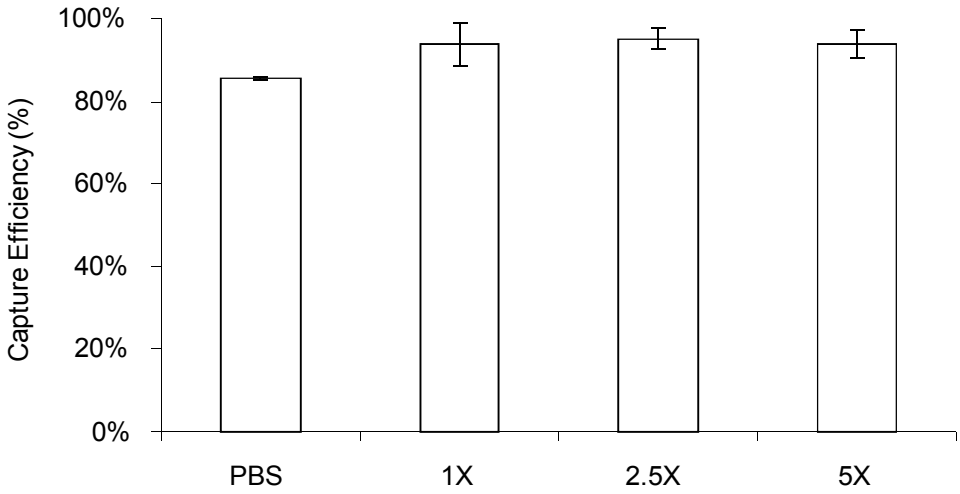


Supplementary Figure 1. Diagram and image of the negative pressure system. (**A**) Schematic of the "negative pressure system" which allows for size based separation of CTCs from whole human blood (red) without complicated or expensive equipment (left panel). Pressure is regulated by a vacuum pump. The "open" filter holder allows for pressure to remain constant, even in the case of plaque buildup. (**B**) Conceptual illustration of filtration based isolation of CTCs is shown (center panel). Smaller red blood cells (red) and white blood cells (yellow) should flow through the filter, while the larger and more rigid CTCs (turquoise) remain on top of the filter. (**C**) Image of the "negative pressure system" used.



Supplementary Figure 2. Capture efficiency of MCF-7 cells in PBS or 1X, 2.5X, and 5X Prefixation Buffer. Cells were pre-stained, enumerated and spiked into 7.5 mL PBS. Samples were then diluted in 7.5 mL of respective buffers, filtered at 5mL/min and enumerated on the filter using a fluorescent microscope. Figures 5A-D shows a typical spiked pre-stained cancer cell after filtration isolation on the filter.

Cell Line Sizes

Cell Line Name	MCF -7	PC3	LnCaP	SKBR3	MB453
Average	**19.1**	**20.8**	**16.0**	**18.1**	**16.1**
SD	**8.4**	**4.8**	**1.8**	**4.7**	**3.7**
Max	**51.3**	**32.2**	**18.7**	**27.4**	**23.9**
Min	**13.4**	**14.0**	**13.4**	**11.3**	**11.1**

Average Capture Efficiency using Immunofluorescent Assay (n=3)

	MCF-7	PC3	LnCaP	SKBR3	MB453
Prefixed Samples	**98±2%**	**95±4%**	**99±2%**	**98±0%**	**96±3%**
Unfixed Samples	**89±6%**	**89±8%**	**89±9%**	**90±4%**	**93±7%**

Supplementary Table 1. Diameters of the 5 cell lines and subsequent capture efficiencies. Prior to testing capture efficiencies (**Figs 2-5)** an undiluted sample from the 5 trypsinized cell lines were each placed on a hemocytometer, 10 randomized cells were picked and their diameters measured by Zen 2011 measurement software. Average diameter, minimum diameter, maximum diameter, and the standard deviation of diameters are shown. (**B**) Capture efficiency of 3 breast cancer cell lines and 2 prostate cell lines using a 160,000 pore design with a 7 µm diameter pore, and the antibody immunofluorescent assay. Enumerated cells were spiked into 7.5mL whole blood with 1X Prefixation Buffer. After wash, filters were fixed, permeabilized and stained with an antibody cocktail specific for CTCs. CTCs were then enumerated based on presence of cytokeratin, EpCAM and DAPI **(Figs 5E-I**). Experiments were run in triplicate.

Pore size	Normal A	Normal B	Normal C	Normal D	Normal E	Average	Average (X100)	Standard Deviation	Standard Deviation (X100)
5	396	545	346	409	609	461	46,104	111	11,087
5.5	288	423	333	278	467	358	35,788	84	8,384
6	90	64	139	71	278	128	12,836	89	8,853
6.5	74	56	30	61	57	56	5,565	16	1,601
7	20	36	23	19	39	27	2,744	9	946
7.5	51	21	26	16	25	28	2,782	14	1,355
8	9	32	35	10	32	24	2,366	13	1,299

Supplementary Table 2. DAPI contamination rate from 5 different normal donors on the 160,000 porosity membranes from Figure 3B. 7.5 mL of whole human blood was diluted in 7.5mL Prefixation Buffer and filtered at 5mL/min. Five images were taken, each representing 1% of the total filtration area, at randomized locations for each filter and averaged. The average counts and standard deviation for each donor is shown.