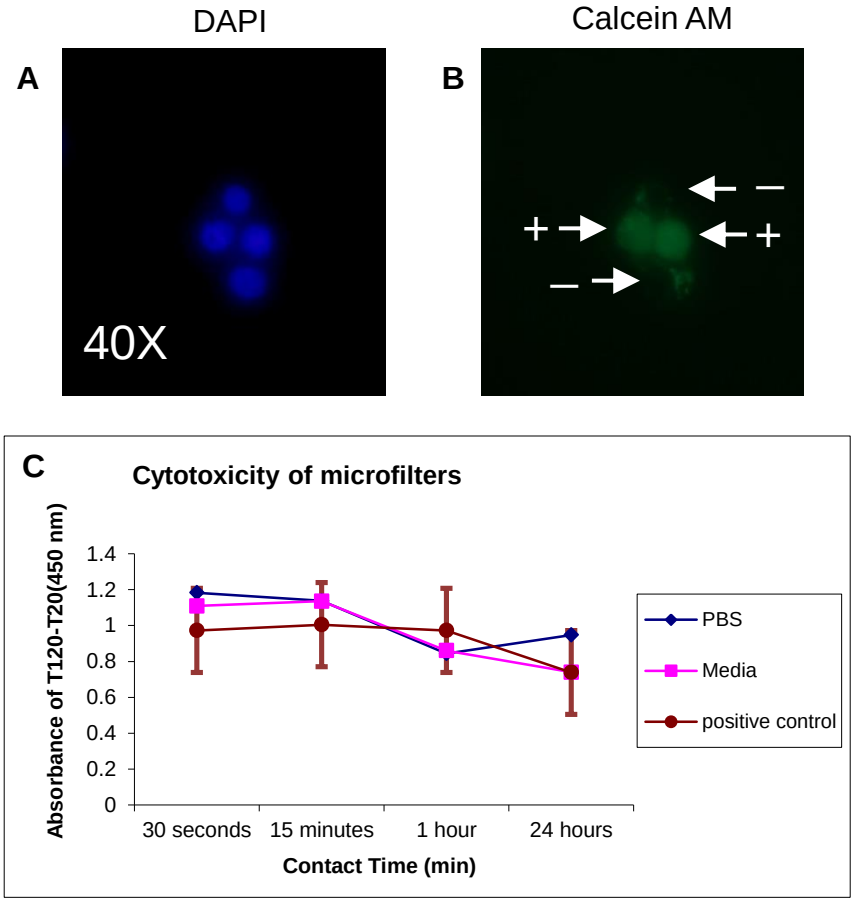
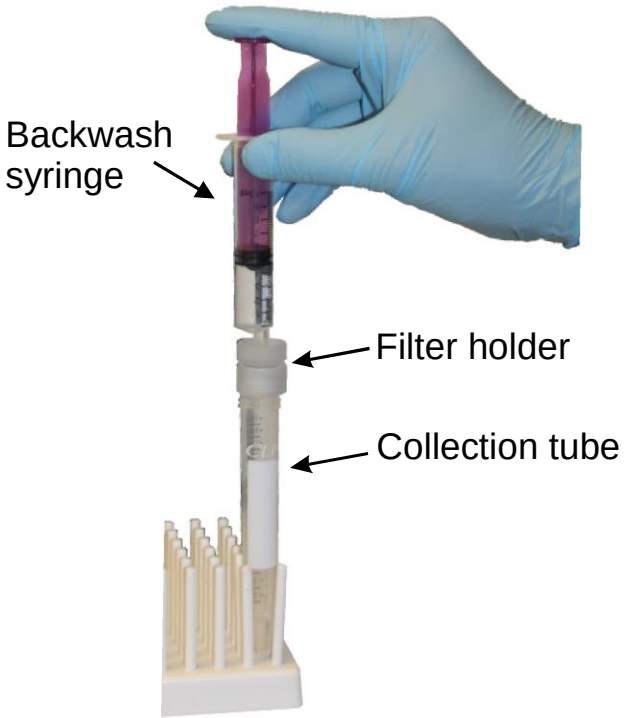
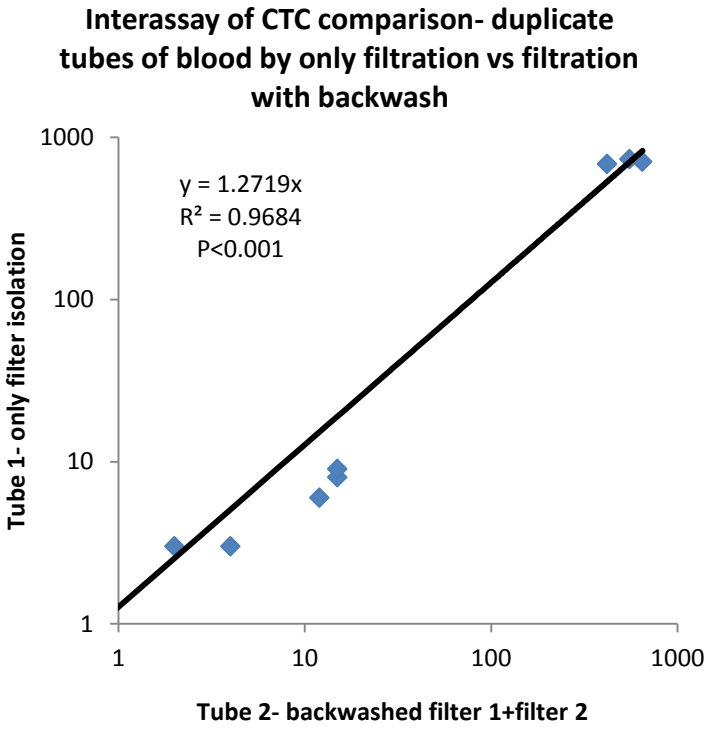
Electronic Supplementary Material (ESI) for RSC Advances.



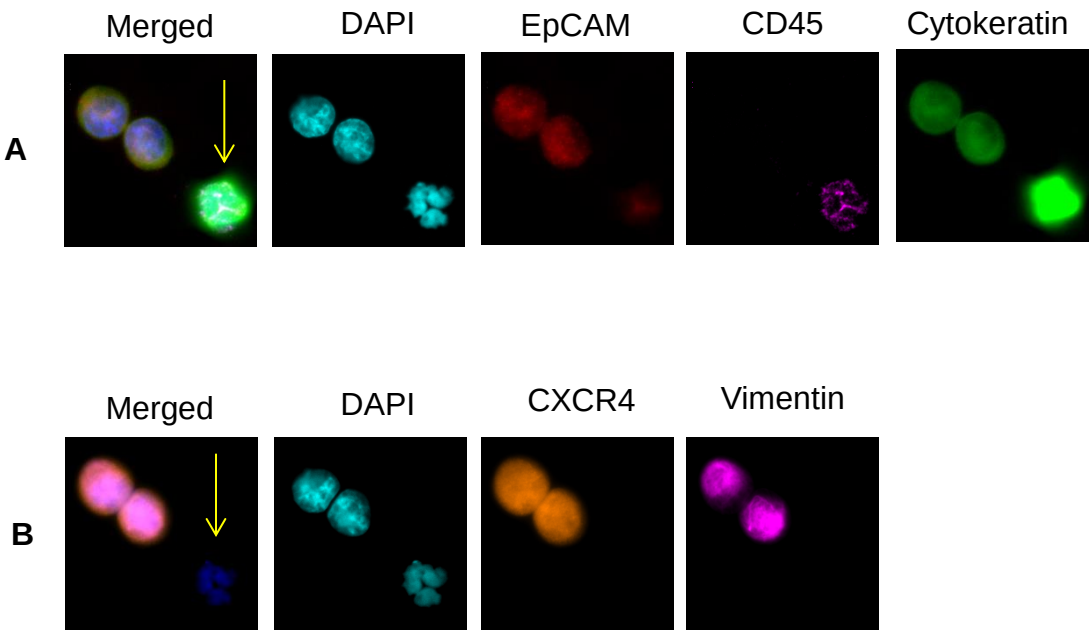
Supplementary Figure 1. Viability of SKBR3 cells in normal blood. SKBR3 cells were spiked into 7.5 ml of K_2EDTA treated normal blood and filtered. DAPI staining shows 4 cells in this cluster. **(B)** Cells were incubated with Calcein AM showing 2 viable cells (+) and 2 dead cells (-). (**C**) Cytotoxicity of the microfilters was assessed by placing 10^6 filter captured MCF-7 cells in 12 well tissue culture plates in the in the presence of either PBS or complete media. As control, MCF-7 cells were plated onto 12 well tissue plates in complete media in the absence of filters. Filters were kept for 30 seconds, 15 minutes, 1 hour or 24 hours in a tissue culture incubator after which cell were counted using a cell counting Kit-8 (CCK-8; Dojindo).



Supplementary Figure 2. CTC backwashed from the filters for downstream analysis. After The filtration process a syringe with PBS is placed on the bottom of the holder. The holder is placed upside down on a collection tube and the PBS “backwashes” the cells into the collection tube.



Supplementary Figure 3. Interassay comparison of CTC isolation versus CTC backwash and isolation. Two duplicate blood tubes from cancer patients were compared by either CellSeive™ microfilter isolation of CTCs or filtration followed by backwash. Figure 6 show that 97% of CTCs were backwashed off the filter and could be recovered. Here we show the number of CTCs captured without backwash is statistically similar to the number of CTCs captured using backwash (n=16).



Supplementary Figure 4. Identifying CTCs and subsequently restaining the CTCs for further subtyping. (A) The assay from Figure 5 was optimized in PANC-1 cell lines by spiking into normal blood and filtering. After identifying and imaging the CTCs and WBCs using the Epithelial stain (e.g. cytokeratin, EpCAM and CD45), the fluorescence was bleached. A CTC doublet can be seen as cytokeratin positive and EpCAM positive, but CD45 negative. A WBC (yellow arrow) can be see as CD45 positive, but also cytokeratin positive. **(B**) The samples were then bleached, as described, and restained with the mesenchymal marker Vimentin and a stromal regulation marker CXC receptor. The WBC was negative for both the markers while the PANC-1 cells were positive for both markers. Scale=72 µm box.