

# ACOUSTOPHORESIS PRE-ALIGNMENT OF CELLS ENABLES LABEL-FREE ENRICHMENT OF PROSTATE CANCER CELLS IN BLOOD

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## ABSTRACT

We have developed a microfluidic chip for isolation of circulating tumor cells (CTCs) in blood based on microchannel acoustophoresis. Ultrasound radiation forces are used to separate cancer cells from blood cells in a continuous flow format. Separation was dramatically improved after incorporation of a 2-dimensional acoustic pre-alignment of the cells before entering the acoustophoresis separation channel. Temperature regulation of the microfluidic chip ( $\pm 0.5^\circ\text{C}$ ) enabled stable and reproducible separation. The chip constitutes a further development and refinement of methods previously presented by our group at  $\mu\text{TAS}$  [1-3].

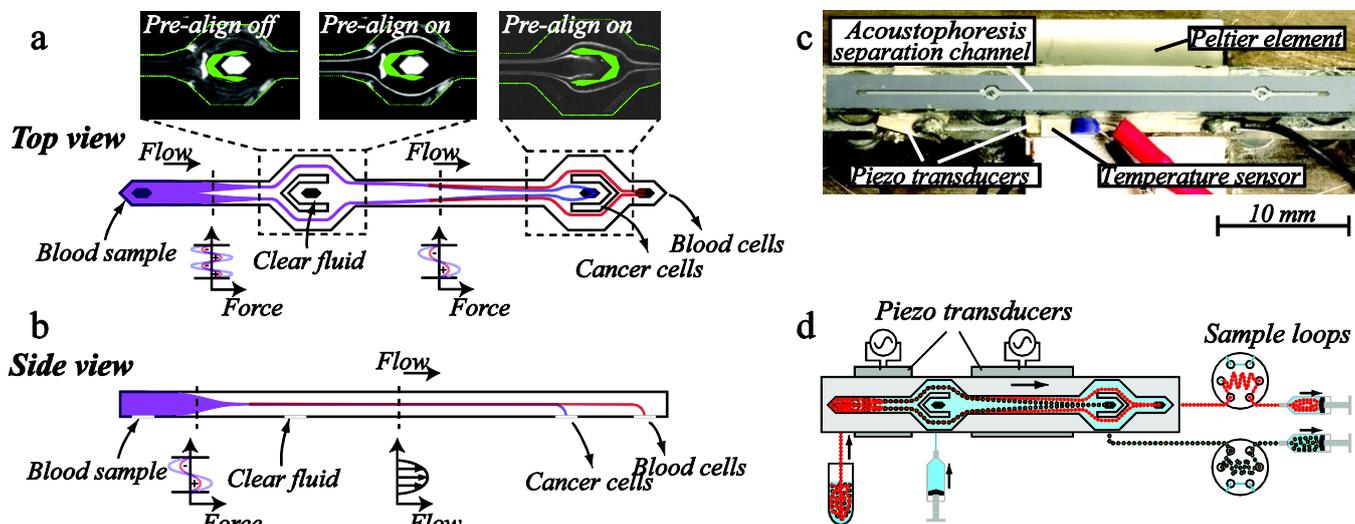
**KEYWORDS:** Ultrasound, Circulating tumor cells, Separation, Blood cells

## INTRODUCTION

According to the world health organization (WHO) the number of cancer related deaths will increase from 8 million in 2008 to 13 million worldwide in 2030. The most common forms are lung-, breast-, colon-, stomach- and prostate cancer, which are all epithelial cancers. Epithelial cancers can form metastases at distant sites through CTCs, which escape the tumor via the blood stream. Isolation of CTCs from patient blood has gained a lot of interest in the recent past since it holds promise for early discovery of metastatic disease.

## EXPERIMENTAL

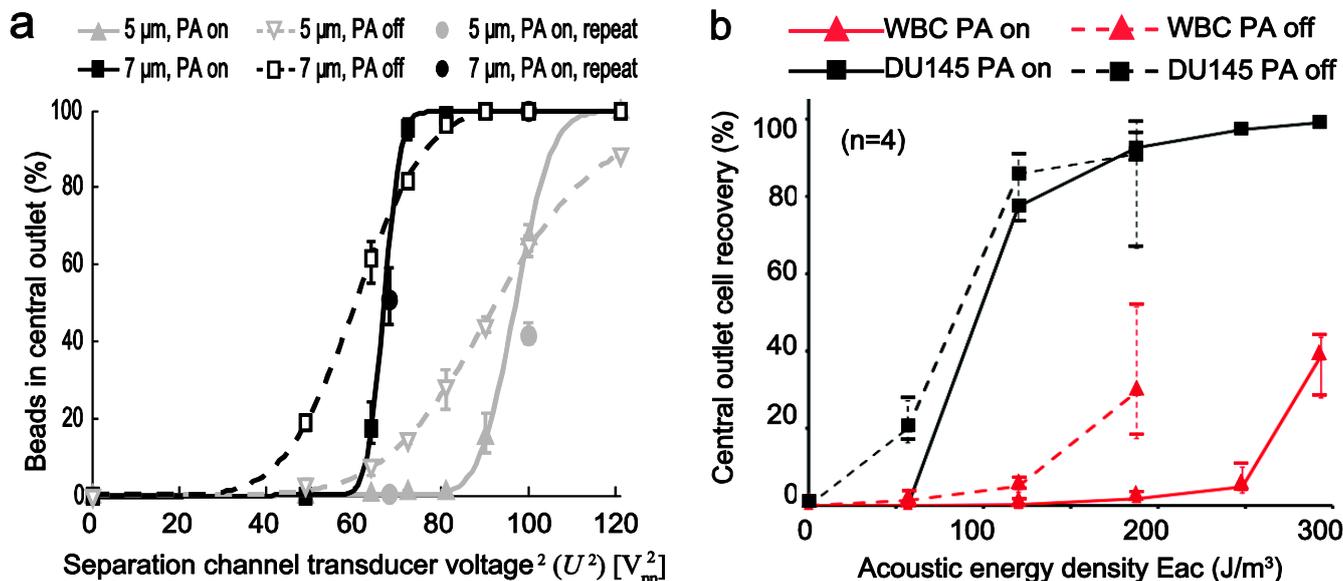
The microfluidic chip separates particles or cells based on size and acoustic properties. Samples of red blood cell (RBC) lysed blood and spiked prostate cancer cells (DU145) were separated in the device. All cells are first pre-aligned in the transverse cross section of the microchannel flow using an acoustic field with components in the height and width dimension, (Fig. 1). Thereafter the cells enter a second channel where separation occurs according to the acoustophoretic mobility of the cells as previously presented [1]. At a trifurcation outlet, cancer cells are collected in a central branch while blood cells are collected in the side branches. To maintain long term stability, temperature control ( $\pm 0.5^\circ\text{C}$ ) has been incorporated in the platform.



**Figure 1.** (a) Cells enter through inlet through a pre-alignment channel by means of an acoustic resonance in the plane orthogonal to the flow. The two bands of cells are bifurcated to two sides of a central inlet flow and the cells are flow laminated to proximity of the walls of a separation channel. Here the trajectories of individual cells are deflected in a second resonant field according to size and acoustic properties. At the trifurcation outlet, cells of high acoustophoretic mobility is guided to the central outlet by tuning the intensity of the resonant field. Cells of low acoustophoretic mobility exit through the side outlet. Insets show pre-aligned and non-pre-aligned microbeads at the end of the pre-alignment channel, and 5 and 7  $\mu\text{m}$  beads separated at the central outlet. (b) Beads are pre-aligned vertically by means of a resonant field to minimize the influence of the parabolic flow profile in the channel, which otherwise affect the trajectories. (c) A photo of the device showing the piezo transducers, the Peltier element for temperature regulation, and the temperature sensor. (d) The flow configuration in the experiments.

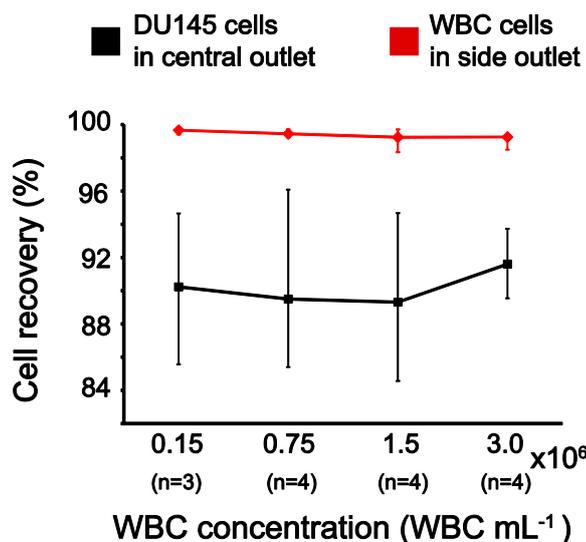
## RESULTS AND DISCUSSION

The acoustic pre-alignment yielded dramatically improved separation as shown in the model system using microbeads of 5 and 7  $\mu\text{m}$ , figure 2a, and for epithelial prostate cancer respectively blood cells in figure 2b. Samples of microbeads 5 and 7  $\mu\text{m}$  were separated in the chip resulting in 99% purity of each bead type at the outlets of the device. Prostate cancer cell recovery in the system was close to 90% while white blood cell contamination was reduced to below 1% at a sample flow rate of 4.2 mL/h.



**Figure 2.** (a) Separation of microbeads. The proportion of 5  $\mu\text{m}$  beads (gray) and 7  $\mu\text{m}$  beads (black) collected in the central outlet, compared to the total number of beads collected, as a function of the piezoceramic transducer voltage squared (i.e. acoustic energy). Acoustophoresis pre-alignment (PA) on (filled symbols) or off (open symbols). The values given are means, the error bars denoting min and max values ( $n=3$ ). (b) Samples of RBC-lysed blood (WBC) spiked with prostate cancer cells (DU145) were processed in the chip. The central outlet cell recovery, i.e. the proportion of cells collected from the central outlet (compared to the total amount of cells collected). The effects of cell pre-alignment (PA) on PFA-fixed cells, DU145 (black), and WBCs (red). Acoustic pre-alignment on (solid lines) and off (dashed lines). The values given are means, and error bars denote max and min, ( $n=4$ ).

To elucidate whether the blood cell concentration affects the cell separation capacity of the acoustic chip, a dilution series of RBC lysed blood was obtained. No significant difference in cancer cell / WBC separation could be detected in the investigated span of  $1.5 \times 10^5 - 3.0 \times 10^6$  WBCs per mL, figure 3. This is an indication that the acoustic chip can process undiluted WBC samples with high accuracy.



**Figure 3.** Tumor cell enrichment using acoustophoretic pre-alignment of cells. Acoustic separation of DU145 cells ( $2.5 \times 10^5 \text{ mL}^{-1}$ ) spiked in different concentrations of WBC at an energy level of  $120 \text{ Vpp}^2$ . The central outlet cell recovery, i.e. the proportion of cells collected from the central outlet (compared to the total amount of cells collected), was measured by flow cytometry.

