Sequential Sorting Approach to Isolate Rare Cells from Whole Blood

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W104e

Abstract

A two stage sequential sorting (2S) microfluidic chip was developed to efficiently isolate rare cells **from whole blood without pre-treatment.** The 2S approach encompasses a *1st stage cell sorting, a longitudinal cell separation (LCS), and a 2nd stage cell sorting region*. The 2S chip isolated greater than 70% of spiked in cultured MDA-MB-231 cells in 8 mL of whole blood. Additionally the enriched isolated spiked cells made up more than 70% of all isolated cells (70% purity).

Background

- Rare cells, such as circulating tumor cells (CTCs) in the peripheral blood of cancer patients, have potential clinical utility for early diagnosis, prognosis, and treatment response monitoring.
- Most microfluidic-based techniques for CTC isolation from whole blood requires pre-enrichment of the blood sample using RBC lysis or Ficoll separation. These enrichment steps often cause a loss of 20 to 50% of the rare cells and are prone to user error and result variability.

Methods

- Fluidic simulations were done with CFD-ACE+.
- Proof of concept testing was done with a bead solution containing 15 μm fluorescent beads as the selection target at a concentration of 100 bead/mL, and 10 μm non-fluorescent beads as the background objects at a concentration of 4.7 x 10^6 bead/mL.
- Analytical validation of the cell isolation and purity was done with about 200 cultured cells added to 8-mL of whole blood and run on the MiSelect R instrument.

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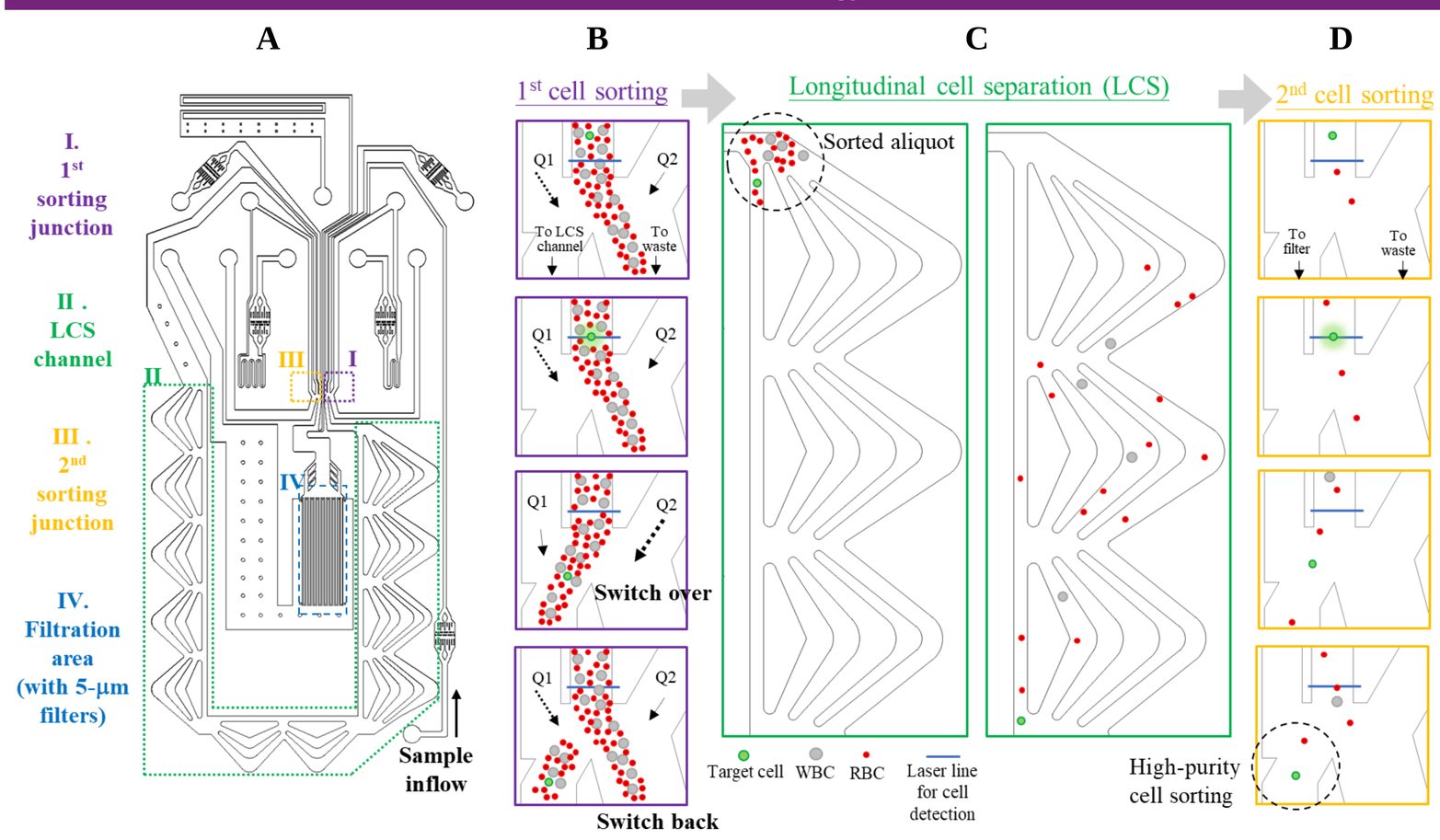
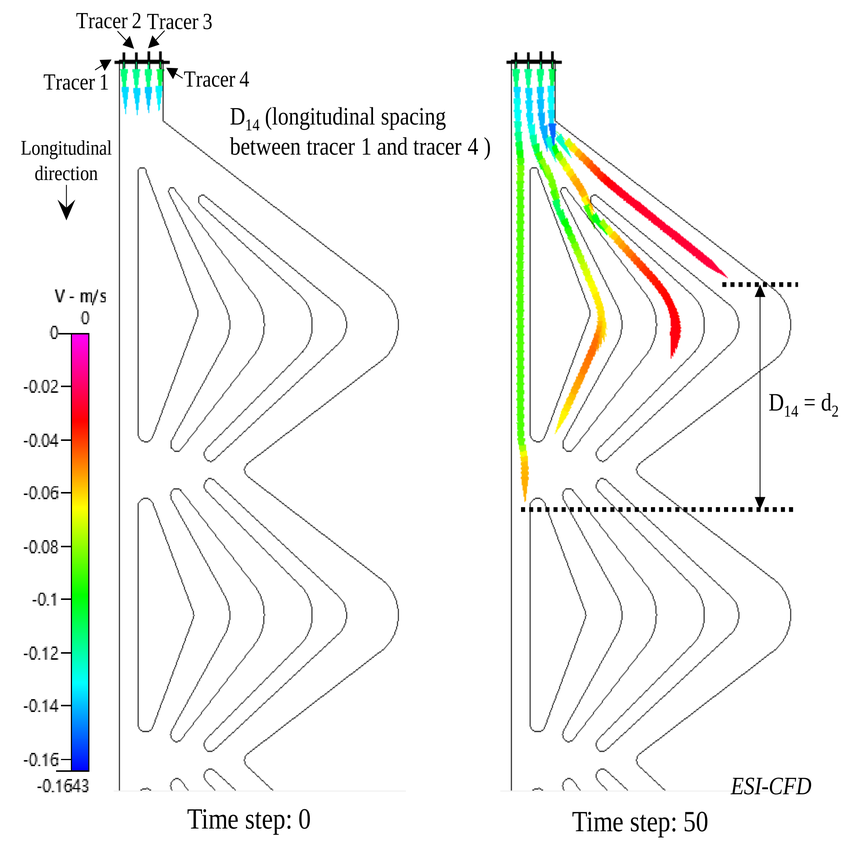
Results

Core Technology



The sample continuously flows through the 2S chip (A). In the 1st sorting stage (B) groups of cells are selected based on laser excited fluorescence. The LCS region spreads out the cells (C) so that the 2nd sorting stage (D) can sort just the target cells with higher purity.

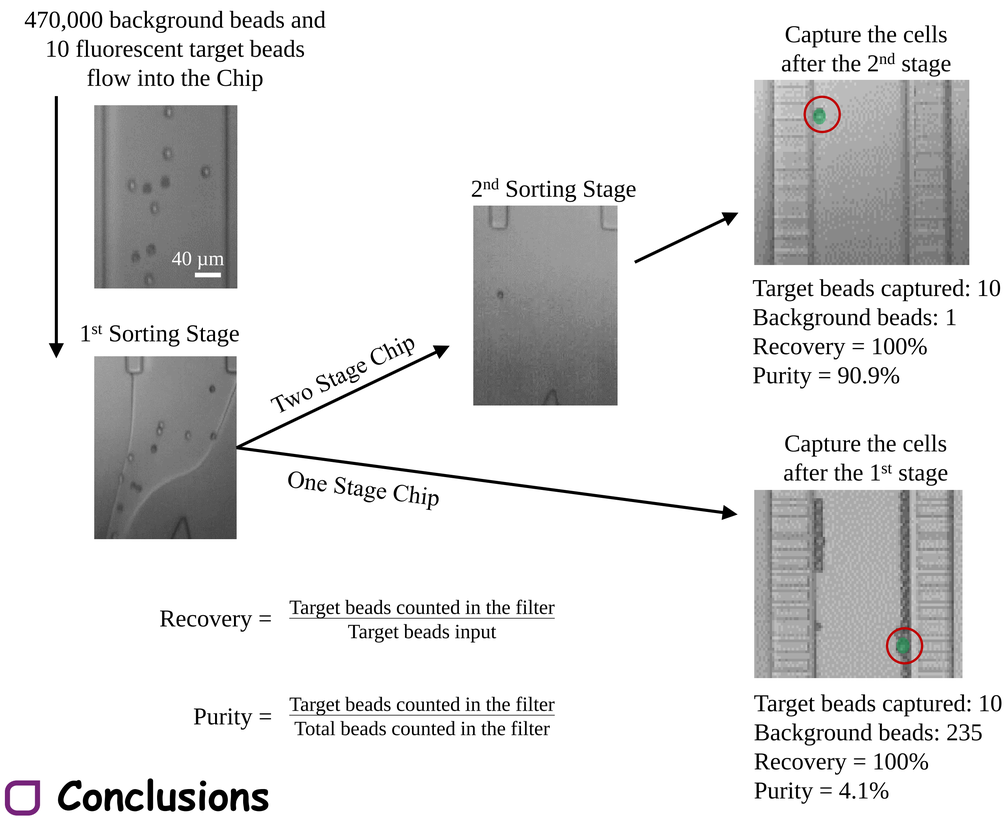
CFD Numerical Simulation



Numerical simulation showing that different fluid lines take different trajectories and travel at different velocities. These differences result in the target cells becoming separated from other non-target cells.

Recovery Test Using a Bead Solution: One Stage vs. Two Stages

Two sorting stages reduced the background beads collected from 235 beads to just one bead



$$\text{Recovery} = \frac{\text{Target beads counted in the filter}}{\text{Target beads input}}$$

$$\text{Purity} = \frac{\text{Target beads counted in the filter}}{\text{Total beads counted in the filter}}$$

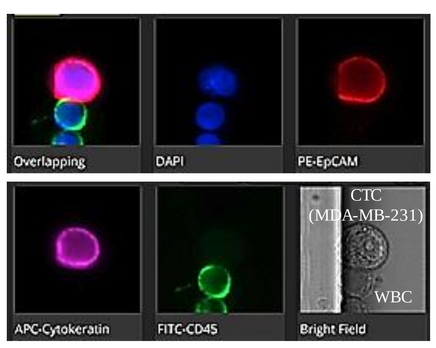
Cell Spike-in Test for VALIDATION

About 200 cultured cells was added to 8 mL of whole blood and incubated with fluorescently conjugated EpCAM antibody

SelectChip microfluidic chip

See Poster No. W094d for the SelectChip design and implementation

The MiSelect R platform is an all-in-one device with a automated process from cell selection, to antibody labeling, to fluorescence imaging



On-chip cell staining for target cell identification

	Cell # added to blood	Cell # captured	White blood cell # captured	Recovery %	Purity %
Test 1	187	135	53	72.2	71.8
Test 2	211	153	26	72.5	85.5

MDA-MB-231 cells in blood sample with **no pre-enrichment** ⇨ **2S Chip** MiCareo Inc. ⇨ **Recovery > 70%** **Purity > 70%**

Conclusions

- The 2S chip recovered > 70% of the low EpCAM cell line MDA-MB-231.
- The recovered cells were isolated with a purity > 70%, with less than 100 white blood cells from 8 mL of whole blood.
- The 2S chip is a promising rare cell isolation approach for single cell analysis and precision medicine.