

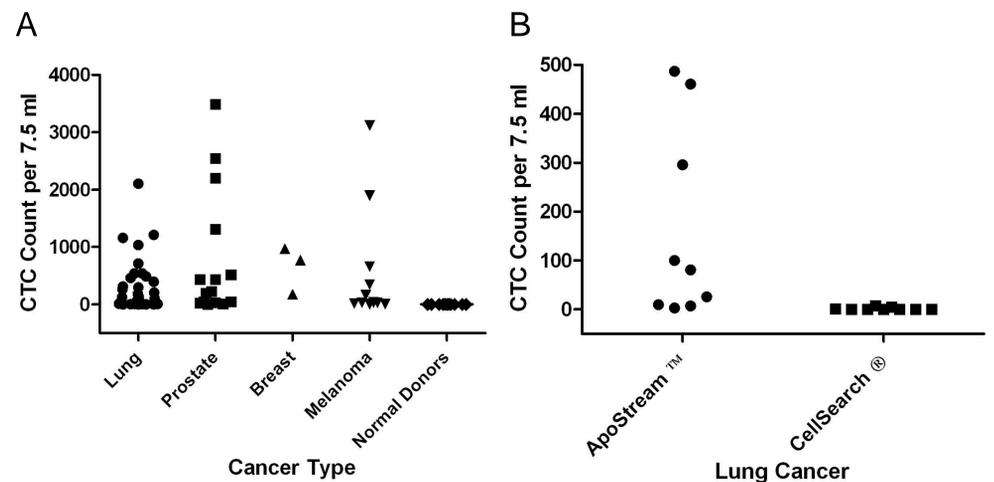
# Antibody-independent enrichment of circulating tumor cells (CTCs) from a variety of cancer types

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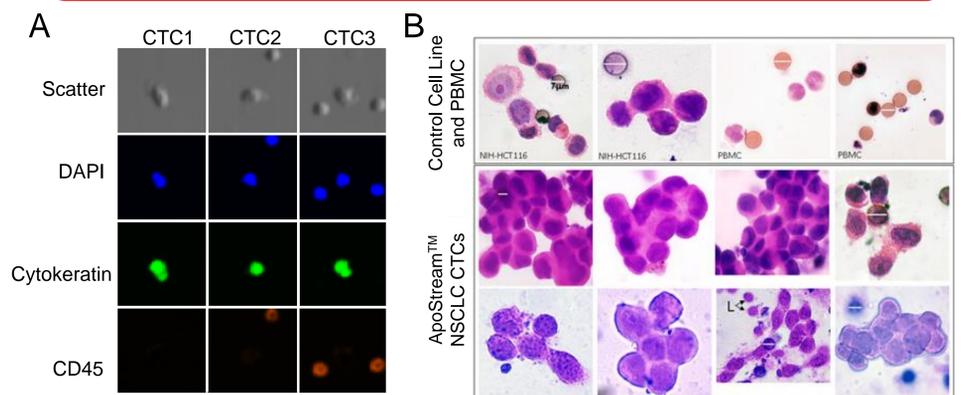
## Abstract

**Background:** Circulating tumor cells (CTCs) are used clinically as biomarkers for monitoring metastatic disease progression. However, CTC capture has been hampered due to the limitations of EpCAM dependent capture methods, non-specific nature of filtration methods and rarity of CTCs. We developed a new CTC enrichment device, ApoStream™, that is based on dielectrophoretic field-flow fractionation (DEP-FFF) in a continuous flow microfluidic chamber enabling antibody independent isolation of viable CTCs. ApoStream™'s DEP-FFF technology leverages inherent differences in cell morphology between normal cells and cancer cells to separate CTCs from other healthy blood cells. **Methods:** To demonstrate performance, ovarian cancer cells were spiked into peripheral blood mononuclear cells (PBMCs) from normal donor blood and isolated using the ApoStream™ device. Cancer cell recovery was demonstrated for cells isolated from the ApoStream™ device from blood of patients with lung, prostate, breast cancer and melanoma. Lung cancer blood samples were compared in a paired sample study with CellSearch®. Cells isolated from ApoStream™ were stained for cytokeratin (CK), CD45, and DAPI, and melanoma CTCs with S100 and CD45, and imaged and enumerated using laser scanning cytometry (LSC). CTC morphology was confirmed with H&E staining. **Results:** ApoStream™ yielded a recovery of  $80 \pm 3\%$  with more than 4000 times enrichment from samples spiked with ovarian cancer cells (SKOV3). High CTC recovery from cancer patient blood samples was achieved with counts ranging from 0 - 2104 (lung, n=33), 0 - 3490 (prostate, n=15), 176 - 968 (breast, n=3), and 4 - 3120 (melanoma, n=11) CTCs per 7.5 ml blood. There were no false-positive CTCs from normal donor blood controls demonstrating ApoStream™'s specificity. In a side-by-side sample comparison with CellSearch® system, ApoStream™ isolated a significantly higher number of CTCs from lung cancer patient blood samples (range: 3-487, mean:163, n=9 versus range: 0-8, mean:2, n=9) showing the effectiveness of ApoStream™ in isolating EpCAM-negative CTCs. In addition, FISH analysis was successfully performed on ApoStream™ enriched cells. **Conclusion:** ApoStream™ technology provides an antibody-independent method for CTC enrichment from various types of cancers with high recovery enabling downstream characterization including protein, RNA and DNA analysis. Isolation of CTCs enriched by ApoStream™ will have broad applications including drug screening, ultimately facilitating implementation of personalized cancer therapy.

## High CTC Recovery using ApoStream™

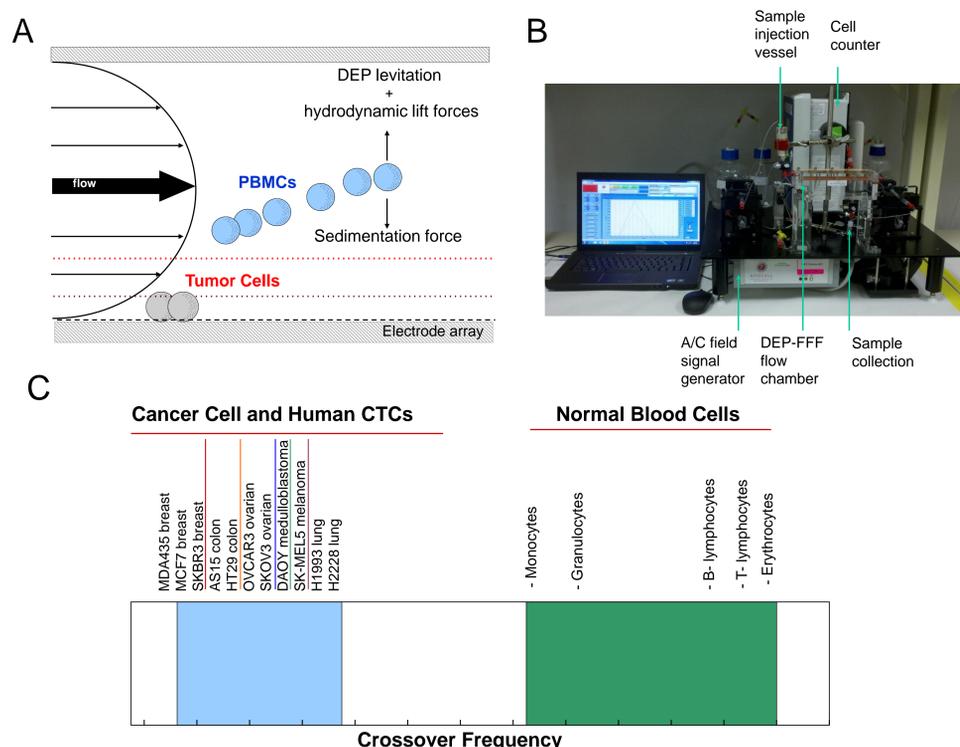


## LSC and H&E Analysis of CTCs

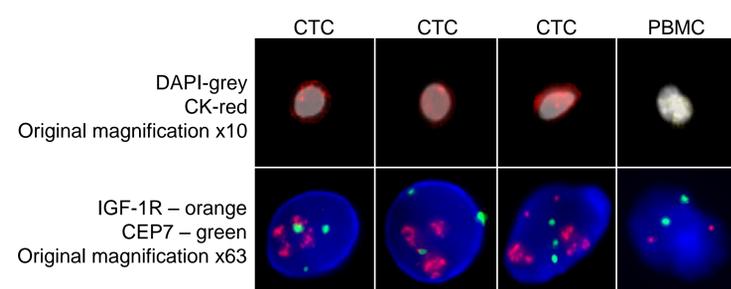


**(A)** CTCs isolated by ApoStream™ from blood of NSCLC patient were identified by immunofluorescent staining as DAPI+/CK+/CD45- cells. **(B)** CTC clusters were isolated from blood of lung cancer patients, as visualized by H&E staining.

## ApoStream™ System



## FISH Analysis



CTCs from the blood of a breast cancer patient have IGF1R amplification. CTCs were isolated by ApoStream™ device, identified by immunophenotyping, and then examined by FISH.

## Conclusions

- ApoStream™ is a high throughput CTC isolation system that permits antibody-independent enrichment of CTCs from various types of cancers with high recovery. ApoStream™ technology isolates higher number of CTCs from patients with NSCLC than the CellSearch® method, evidently due to capturing EpCAM-negative cancer cells.
- CTC capture with ApoStream™ allows downstream CTC characterization such as protein expression, gene expression, mutation analysis and FISH.
- ApoStream™ can serve as a new effective tool with broad applications in cancer biomarker discovery and implementation of personalized cancer therapy.

## Acknowledgments

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