Detection of Circulating Rare Cells through an integrated Inverse Magnetophoresis Enrichment method and Image-Enhanced Flow Cytometry

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Introduction

Circulating tumour cells (CTCs) harbour intact molecular signatures of their DNA, RNA and protein cargo, making them an attractive tool for monitoring cancer and treatment decision-making [1,2]. However, isolating and characterising these rare and heterogeneous CTCs, particularly those undergoing epithelial-mesenchymal transition (EMT), remains challenging [3,4]. Current CTC isolation techniques often suffer from high costs, lack of scalability and contamination with white blood cells (WBCs) [5].

Objectives

We developed an automated workflow that integrates the KingFisher Apex™ system with the Attune CytPix™, an Invitrogen™ flow cytometer designed for the detection of rare events. The KingFisher Apex™ system simultaneously utilised two CTC isolation approaches: an EpCAM-Dynabead-based positive isolation method (*P-SeLect*) and a CD45-Dynabead-based negative isolation method (*N-SeLect)* through inverse magnetophoresis.

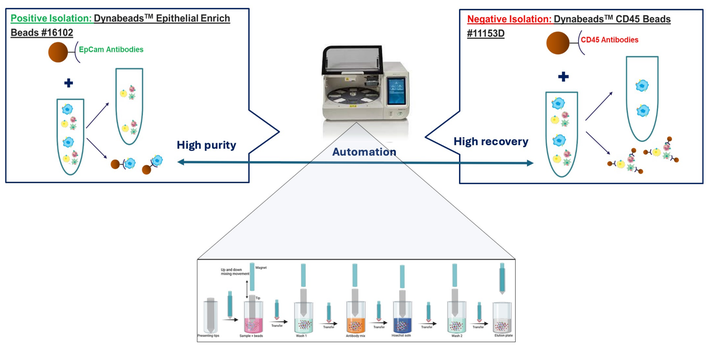

Figure 2:Positive and Negative isolation workflows on the KingFisher Apex™. Co-developed workflow for both positive and negative isolation of CTCs that can be taken forward for cell culture, immunostaining or direct lysis for molecular profiling

Methods

We evaluated the performances of the P-SeLect and N-SeLect workflows for CTC enrichment using breast cancer (BCa) cell line models, focusing on cell recovery rates, WBC depletion and CTC purity. The BD FACSAria™ Fusion flow cytometer was utilised for cell sorting and Attune™ CytPix™ was used for precise cell counting to allow direct comparison between the two platforms.
Subsequently, defined numbers of green-fluorescently labelled BCa cell lines MCF-7 and MDA-MB-231 cells were spiked into healthy peripheral blood mononuclear cells isolated from nine health donors using Ficoll-Paque PLUS. Quantitative RT-PCR (qRT-PCR) was used to measure the expression of EpCAM and TROP-2, a transmembrane glycoprotein broadly expressed in all BCa subtypes.

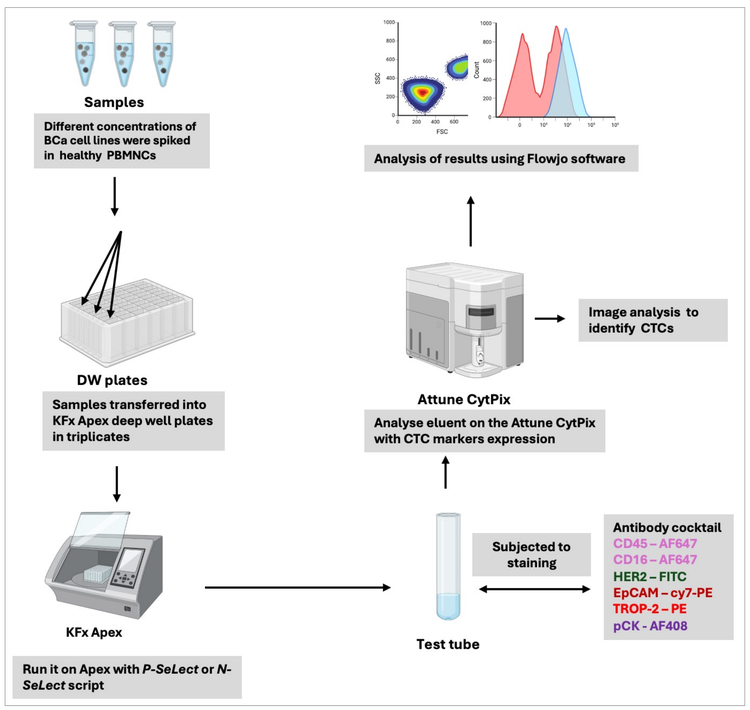

Figure 2: Experimental workflow for the integration of the positive and negative isolation on the KingFisher Apex™ with the Attune CytPix™

Results

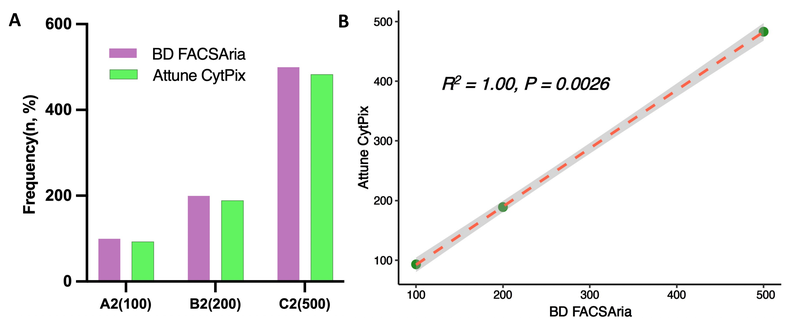

Figure 3: Correlation of cell sorting using the BD FACSAria Fusion™ flow cytometer and cell counting with the Attune™ CytPix™ flow cytometer. **(A)**Comparison of cell sorted and counts on the two flow cutometer platforms. (**B**) Attune™ CytPix™ yielded cell counts highly correlated with those sorted with the FACSAria Fusion™ (R^2 =1.00, p=0.003).

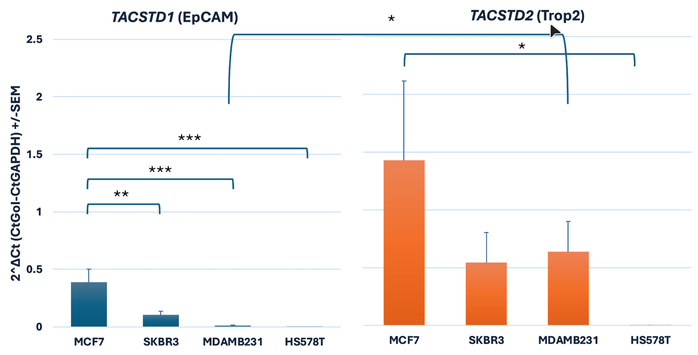

Figure 4: Quantitative RT-PCR measurements of EpCAM and TROP-2 expression across BCa cell lines. TROP-2 expression was consistently higher than EpCAM across cell lines, with the mesenchymal-like MDAMB- 231 cell line showing minimal EpCAM expression

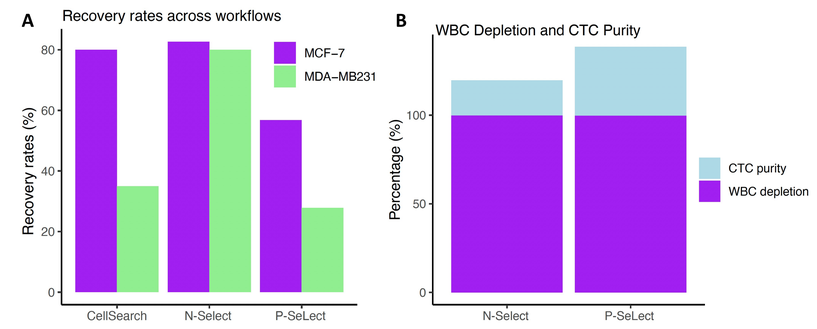

Figure 5: Evaluation of recovery rate of spiked CTCs, WBC depletion and CTC purity. (A) *N-SeLect* demonstrated superior recovery rates (82.7% for MCF-7 and 80.0% for MDA-MB-231) compared to *P-SeLect* workflow (56.8% for MCF-7 and 27.8% for MDA-MB-231). In contrast, the FDA-approved EpCAM-based CellSearch workflow recovered 80% of MCF-7 but only 35% of MDA-MB-231. **(B)** N-SeLect recorded >99% WBC depletion and a CTC purity of 19.8% whereas P-SeLect achieved a higher purity of 38.7% at the cost of lower recovery rates.

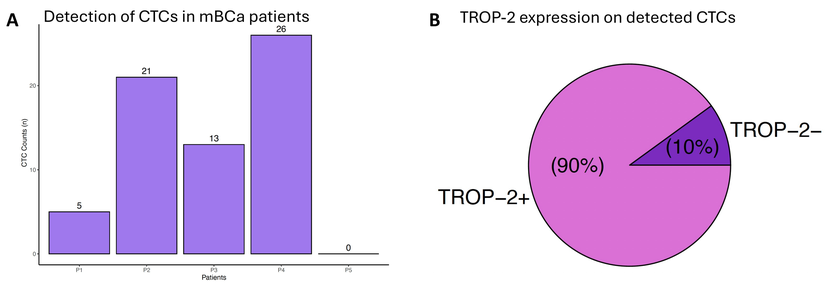

Figure 6: Validation of CTC enrichment and detection method in metastatic BCa patients. (A) CTCs were detected in 80% of cases (4/5) with a median count of 13 cells (range: 5-26). **(B)** Notably, 90% of detected CTCs expressed TROP-2, underscoring the workflow's ability to capture clinically relevant CTC subtypes

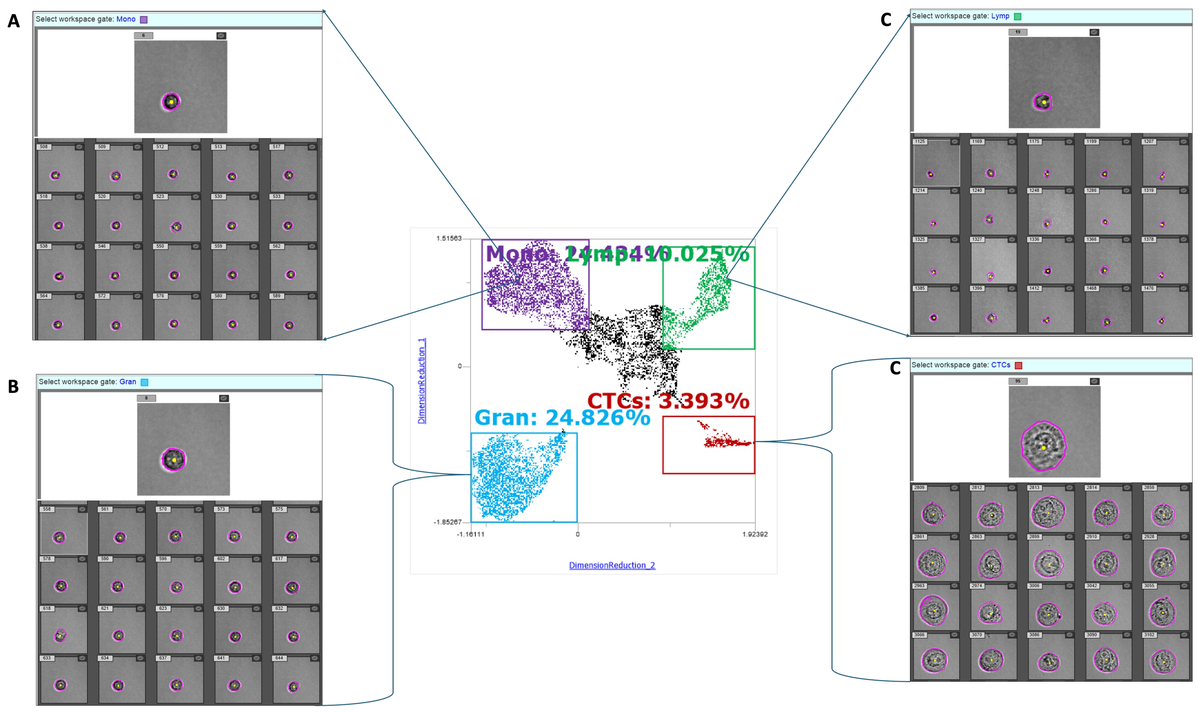

Figure 7: Flow cytometry profiles of MCF7 spiked in healthy total PBMNCs.
We applied dimension reduction using Uniform Manifold Approximation and Projection (UMAP) to visualise and analyse high-dimensional flow cytometry data to identify distinct rare cell populations based on cell size. UMAP analysis with images of spiked MCF7 in Health PBMNCs. DimensionReduction1 (UMAP1) and DimensionReduction2 (UMAP2) are non-linear embeddings of the flow data based on cell size. **(A)** represents monocyte cluster with images **(B)** represents granulocyte cluster with images **(C)** represents lymphocyte cluster with images, **(D)** represents spiked-in MCF7 (CTCs) cluster with images

Conclusion

Our proof-of-concept integrated workflow combines high-efficiency CTC enrichment via inverse magnetophoresis with multiparametric image-enhanced flow cytometry for detailed analysis. It offers a scalable, cost-effective platform for isolating and characterising heterogeneous CTC populations with high recovery rates and minimal WBC contamination. This innovative approach holds significant promise for advancing liquid biopsy applications, enabling real-time cancer diagnostics and informing personalised treatment strategies in clinical settings.

References

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