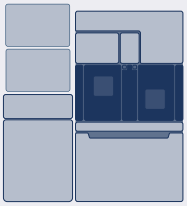
DRUG DISCOVERY

THINKCYTE PHOREMOST

Intracellular Protein Aggregation as a High Content Readout in Flow-Based Phenotypic Drug Discovery



About VisionSort

ThinkCyte's VisionSort platform combines the strengths of conventional flow cytometry fluorescence signals with a novel morphometric cellular analysis measure. The dual-mode analytical capability can be used to identify and sort phenotypically defined cell populations, label free, using machine learning approaches.

INTRODUCTION

Phenotypic drug discovery (PDD) is a powerful approach for identifying new drug targets and elucidating disease mechanisms. Current approaches for PDD mainly rely on either microscopy-based or flow cytometry-based technologies for analyzing readouts. Microscopy-based screening platforms are well established, but are limited by their low throughput and resource-intensive infrastructure. On the other hand, conventional flow-based screening platforms allow for higher efficiencies and large-scale screening, but only work for a limited number of simple phenotypic readouts with defined markers. For many diseases, including cancer, infectious diseases, neurodegenerative diseases, etc., complex phenotypes that involve morphological transformation or intracellular dynamics are important readouts for drug screening.

Here we introduce a new approach for flow-based high content phenotypic screening by combining ThinkCyte's AI-enabled VisionSort platform powered by Ghost Cytometry (GC) with PhoreMost's PROTEINi® technology, targeting intracellular protein aggregation as the target readout. GC is a novel flow cytometry technology that enables analysis and sorting of cells based on both label-free morphological features and conventional fluorescence parameters with high intracellular spatial resolution. PROTEINi is capable of probing the entire proteome to systematically unmask new and unanticipated druggable sites, directly linking them to useful therapeutic functions. When used together, GC and PROTEINi have the potential to rapidly screen for a range of new, complex phenotypes and unmask truly novel drug targets for treating diseases.

Visit ThinkCyte.com to learn more about **VisionSort**, the world's first dual mode, AI-driven cell characterization and sorting platform.

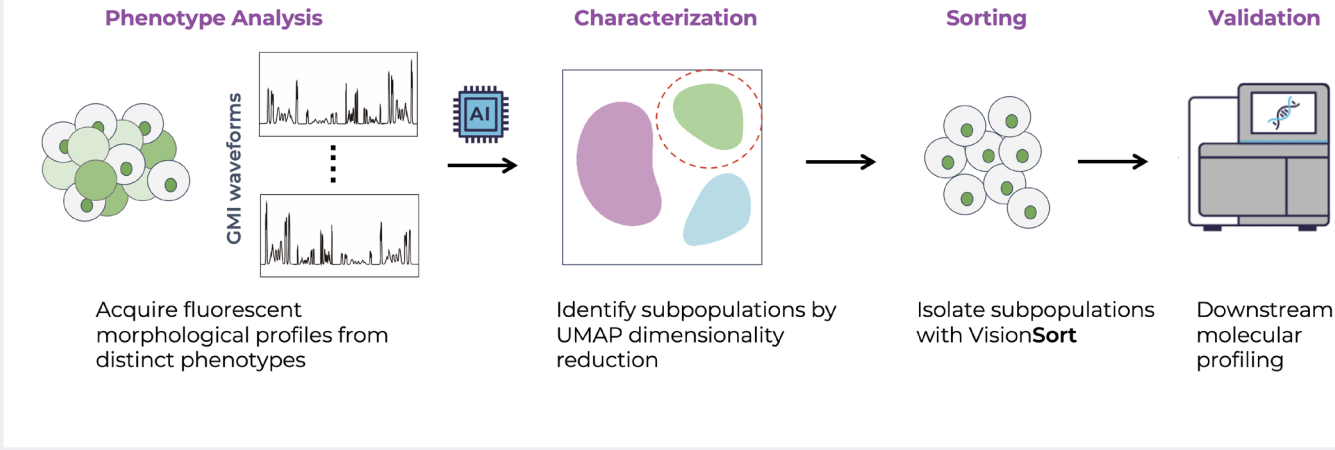


Figure 1. Workflow for unsupervised classification and isolation of target cells using the VisionSort platform. Morphological information of each cell is acquired as a compressed temporal waveform (GMI waveforms) using Ghost Cytometry. Individual cells were mapped onto a two-dimensional space using dimensional reduction methods on the waveforms for visualization and identification of subpopulations of interest. Target subpopulations were gated in the UMAP space and used to create classification models. Target cells isolated by this approach can be used for downstream analysis, such as multi-omics analyses and functional assays.

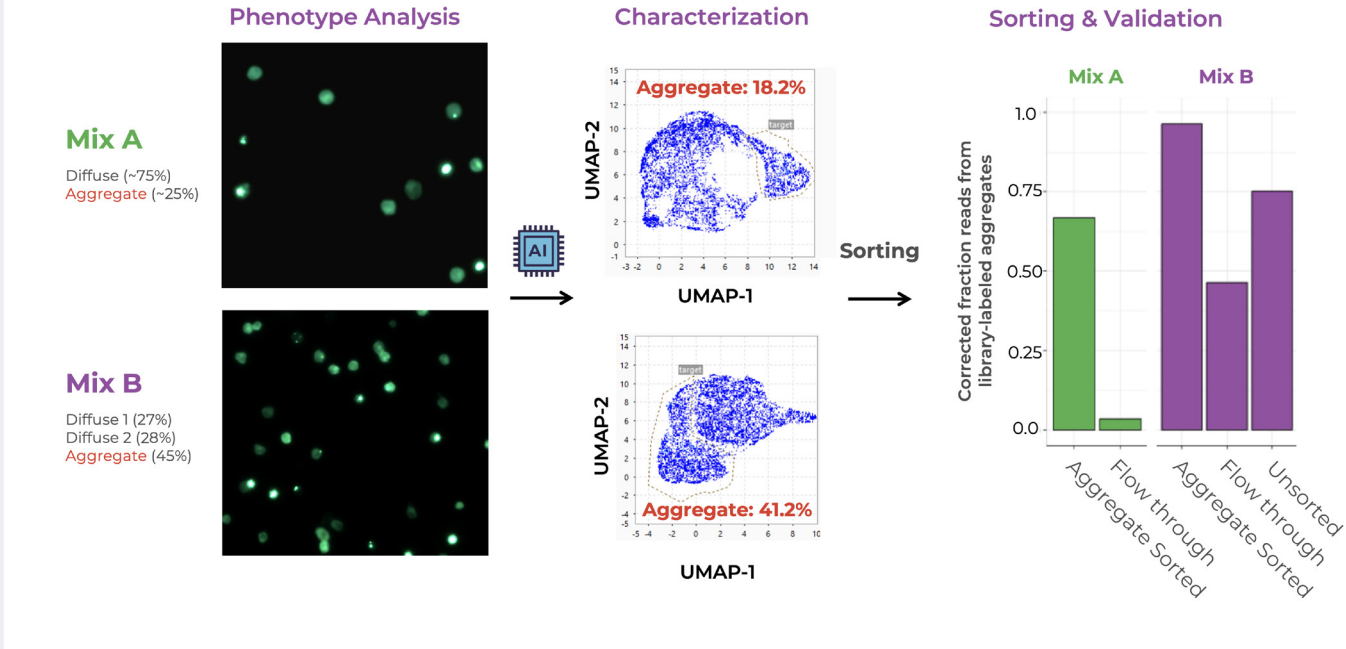


Figure 2. Unsupervised learning approach & sorting validation. MCF10a cell lines expressing Dox-inducible GFP-tagged proteins were mixed in different proportions of aggregation and diffuse phenotypes (Mix A in green and Mix B in magenta). Mix B contained two distinct forms of the diffuse phenotype (Diffuse 1 and 2). Unsupervised machine learning classification of the cell populations was performed on VisionSort. Multiple cell populations were identified in the UMAP space as observed by distinct clustering patterns. Target cells based on phenotype (red gate for the aggregated phenotype) were identified by gating, collected for enrichment and validated using NGS readouts of DNA barcodes.

RESULTS

We prepared an MCF10a cell line model that expressed Dox-inducible aggregation of GFP-tagged proteins. Cells were labeled with unique DNA barcodes based on their intracellular protein aggregation phenotypes, either diffuse or aggregated, and were mixed together for analysis via GC (**Figure 1**). To identify and classify the different phenotypes, we captured the intracellular spatial distribution of the GFP signals as an optical waveform and performed dimensionality reduction using UMAP to visualize and identify unique clusters represented by the phenotypes. 20 million cells were analyzed and sorted in 2 hours based on their UMAP clustering with maintenance of cell viability and integrity. Downstream deep sequencing analysis validated that the enriched cells indeed contained DNA barcodes for the aggregated phenotype (**Figure 2**).

SUMMARY

VisionSort was able to identify and differentiate cells expressing different intracellular protein aggregation phenotypes in high throughput. The approach was able to screen cells with high purity and maintain viability for downstream analysis and validation, demonstrating the applicability of VisionSort for high content phenotypic screening drug discovery programs.

ACKNOWLEDGEMENTS

This work was performed in collaboration with PhoreMost Ltd. For more information on PhoreMost and their SITESEEKER® drug target identification platform visit phoremost.com.

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