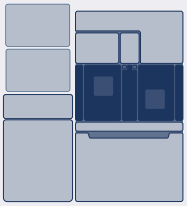
DRUG DISCOVERY

THINKCYTE

Classification of mitochondrial morphology in activated T cells



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. Microscopy-based screening platforms are well established and obtain high content image information, however the current approaches lack versatility, are subject to well-to-well variation, and require time-intensive image processing and analysis. Conventional flow-based screening platforms allow for higher efficiencies and larger scale screening, but do not provide high content morphological information about cell phenotypes and only limited fluorescence resolution.

Here we introduce a new approach to flow-based high content phenotypic screening using the VisionSort platform, powered by Ghost Cytometry (GC). GC is a novel flow cytometry technology that enables analysis and sorting of cells based on 1) conventional general fluorescence, 2) detailed spatial fluorescence, and 3) label-free morphological features. Changes in T cell states such as differentiation and activation, are correlated with changes in mitochondrial morphology which is a common phenotype used in drug screening campaigns. Here we show the application of GC's unique high-resolution spatial fluorescence capability to detect mitochondrial morphological changes in activated human T cells.

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

RESULTS

To induce T cell activation, frozen Pan-T cells were thawed and cultured with CD3/CD28 Dynabeads and IL-2 for 2 days. The cells were then cultured for an additional 2 days with IL-2. Non-activated T cells were cultured with only IL-2 for 4 days. After incubation, stimulated and unstimulated cells were mixed, stained with BioTracker 405 blue mitochondria dye, PE anti-human CD3, APC anti-human CD25, FITC anti-human CD69, and Zombie NIR fixable viability kit. The cell mixture was analyzed using both a conventional flow cytometer and Visionsort (**Figure 1**). The changes in mitochondrial morphology were subtle (**Figure 1A**) and it was difficult to separate the two phenotypes based on total fluorescence intensity using a conventional flow cytometer (**Figure 1B**). To classify the different mitochondrial morphology using VisionSort, we captured the intracellular spatial distribution of mitochondria dye as an optical waveform and used it to train a supervised machine learning classifier. The resulting classifier showed excellent performance and reproducibility in separating activated from non-activated T-cells with an AUC = 0.992 +/- 0.002 (n=3)(**Figure 1C**). We also performed unsupervised machine learning using dimensionality reduction on the VisionSort-generated optical waveforms using Uniform Manifold Approximation and Projection (UMAP) to classify unique clusters represented by mitochondrial morphology. We observed distinct populations of activated and non-activated T cells on UMAP (**Figure 2A**). In addition, we observed a gradient of T cell activation markers (CD25 and CD69) on UMAP, allowing VisionSort to visualize the relationship between mitochondrial morphological changes and T cell activation status (**Figure 2B**).

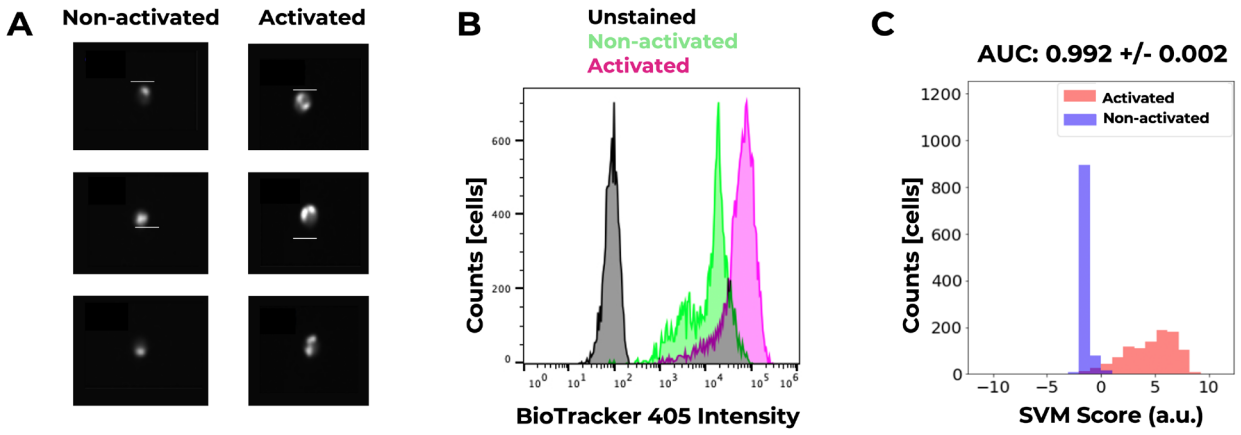


Figure 1. Classification of mitochondrial morphology in non-activated and activated primary human T cells using supervised machine learning. (A) Mitochondrial morphological changes in activated and non-activated primary human T cells. Representative T cell images obtained using Amnis Image Stream (20X objective) are shown. Scale bars = 10 um. **(B)** Total fluorescence intensity of mitochondria dye in unstained, non-activated, and activated T cells. **(C)** Results of supervised machine learning classification on VisionSort. Classification performance is displayed as histograms of support vector machine (SVM) scores, where red and blue colors correspond to ground truth labels for activated and non-activated populations, respectively. 2,000 cells were used to train the models and. 1,000 cells were used for model testing. AUC= Area Under the Curve.

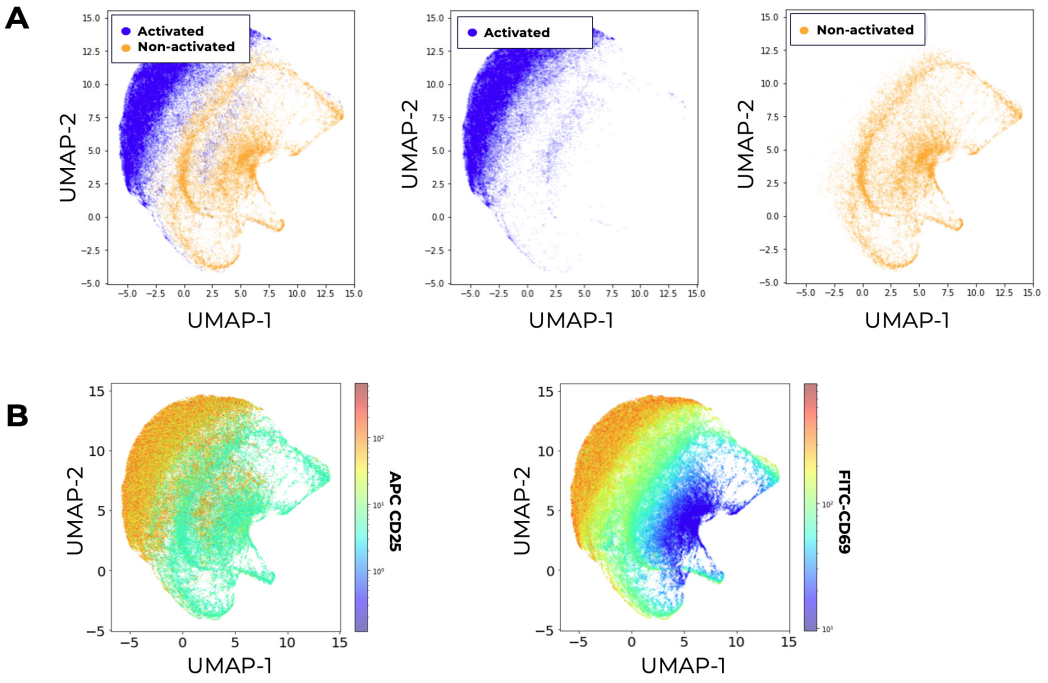


Figure 2. Visualization of mitochondrial morphological diversity in T cells by Uniform manifold approximation and projection (UMAP). (A) UMAP of T cell populations using high-resolution spatial fluorescence profiles generated on VisionSort as a classification feature. Known T cell identity (activated (blue) and non-activated (orange)) as identified by ground truth labels are overlaid in color. **(B)** T cell activation markers (CD25 and CD69) are overlaid onto UMAP.

SUMMARY

VisionSort was able to identify and classify mitochondrial morphological changes depending on T cell activation using both supervised and unsupervised machine learning approaches. Using the high-resolution spatial fluorescence mode on VisionSort, users can identify fine intracellular morphological changes which can be leveraged for phenotypic drug discovery campaigns and disease profiling.

THINKCYTE INC.

UNITED STATES
1100 Island Drive, STE 203
Redwood City, CA 94065

JAPAN
7-3-1 Hongo,
Bunkyo, Tokyo

CONTACT@THINKCYTE.COM **WWW.THINKCYTE.COM**