Label-free morphometric characterization of T cells for cell and gene therapy research and development

THINKCYTE

205

Keisuke Wagatsuma[1], Mika Uematsu[1], Kazuki Teranishi[2], Asako Tsubouchi1, Yuri An[1], Ryo Tamoto[1], Yuichi Yanagihashi[1], Yoko Kawamura[1], Yuri Murata[1], Satoru Akai[1], Kaoru Komoriya[1], Hiroko Nomaru[1], Andy Wu[2], Pj Chana[2], Sadao Ota[1,3]

[1]ThinkCyte KK, Tokyo, Japan | [2]ThinkCyte Inc, Redwood City, CA | [3]Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan

Abstract

Here we used VisionSort, a new label-free cellular analysis and sorting platform built with proprietary optics and artificial intelligence (AI), to characterize and isolate truly untouched human T cell subsets. VisionSort was used to identify T-cells with therapeutically relevant phenotypes, an emerging need in cell therapy R&D. By capturing single-cell digital phenotypes, we characterized human T-cells and generated 'ground truth' functional profiles for 1) glycolysis level, 2) exhaustion state 3) activation or resting profiles and 4) viability. We show that using both supervised and unsupervised machine learning approaches, we could discriminate between specific, therapeutically relevant, T-cell phenotypes.

Introduction

Cell and gene therapies (C&GT) are a novel category of biological medicines where active research and development (R&D) is helping researchers and clinicians understand what makes for the most efficacious therapies. With T cell-based therapies leading the therapeutic product pipeline, identification, characterization, and minimally invasive isolation of T cells is critical for understanding how to best develop new cellular therapies and manage disease. Modern T cell cellular immunotherapy approaches demand new technologies that can isolate and characterize T cells and their functional subsets in their native biological and functional states to improve the quality and efficacy of cell therapy drug products.

Methods

We used VisionSort, a new label-free, artificial intelligence (AI)-driven cellular analysis and sorting platform, to isolate and characterize truly untouched human immune cell subsets for downstream R&D applications. VisionSort is powered by Ghost Cytometry, a technology that combines label-free morphometric profiling with AI-based cell analysis and sorting. The main functional features of Ghost Cytometry are shown in **Figure 1**.

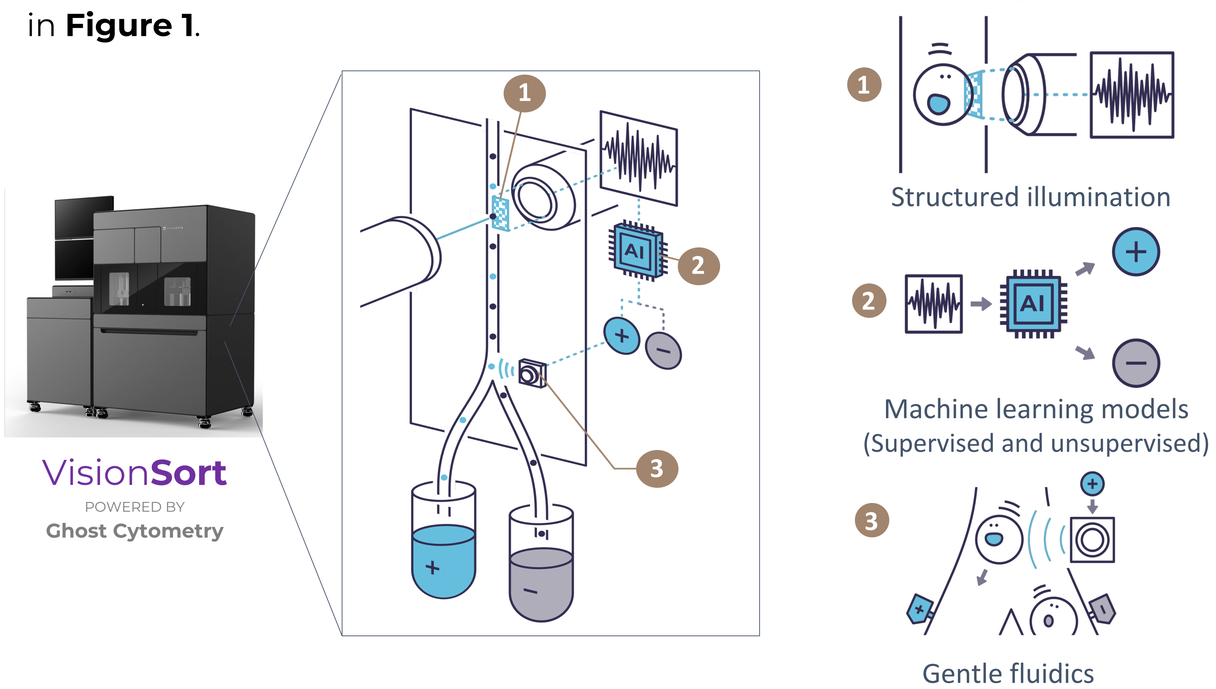

Figure 1. Principles of the VisionSort platform. Individual cells pass through a structured illumination (1). Scattered light profiles (reflecting cell morphology) together with fluorescence readouts from individual cells are used together with embedded AI in the instrument (2) to generate machine learning models that associate target cells with specific morphological profiles. When unlabeled cells are run, the instrument identifies target cells using morphological profiles alone and isolates cells label-free using gentle fluidic pressure (3).

Results

A set of machine-learning derived classifiers, trained on truth sets, was generated to identify these phenotypic classes, or biological states, in unlabeled T-cell subsets. The classifiers showed area under the curve (AUC) performance ranges for detecting specific T-cell populations/states between 0.916 and 0.993. Next, we explored the use of VisionSort to identify distinct T cell subsets based on their morphology in a label-free manner. Using a combination of supervised and unsupervised machine learning approaches, we show that we can identify follicular helper T (Tfh) cells from other CD4+ T cells using a supervised approach. Using an unsupervised approach, we analyzed the morphological profiles of T cell subsets using a uniform manifold projection and approximation (UAMP), we found this high dimensional clusters method shows gamma delta (γδ) T cells form skewed clusters from their alpha beta (αβ) counterparts.

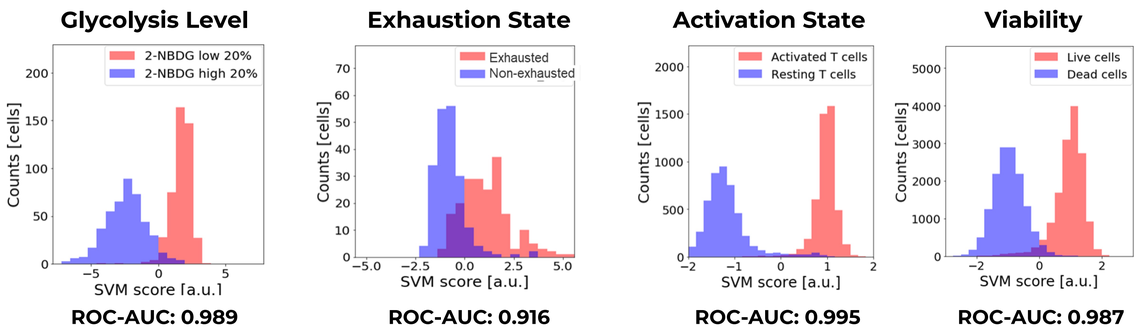

Figure 2. VisionSort for label-free characterization of T cell phenotypes. Performance of machine learning classifiers developed for specific T-cell functional phenotypes on unlabeled test cells.

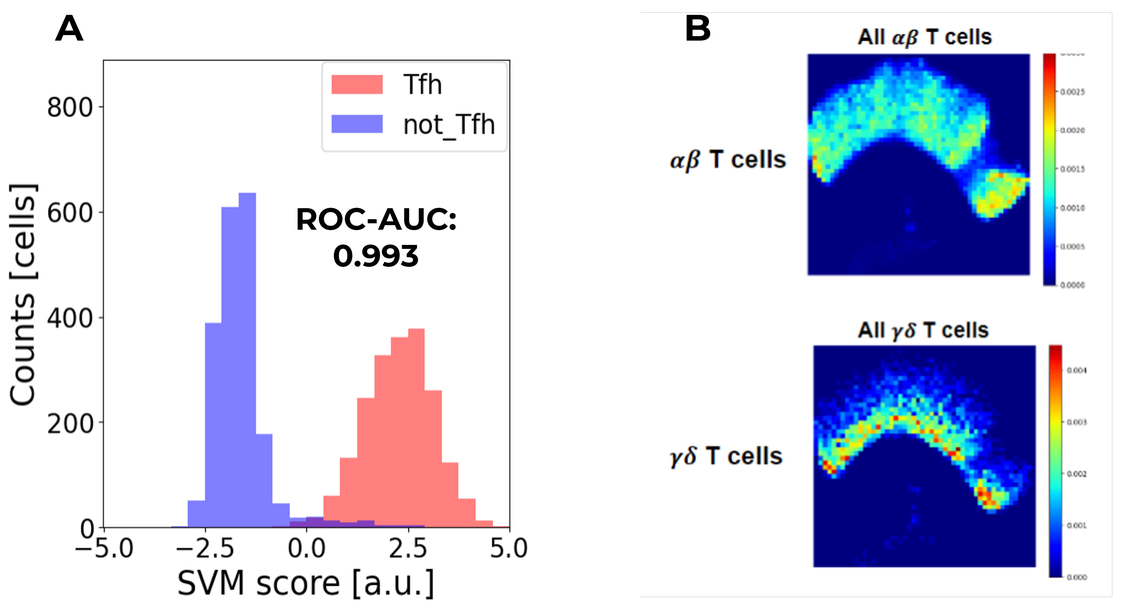

Figure 3. VisionSort for label-free identification of functionally distinct T cell subsets. (A). Follicular helper T (Tfh) cells were able to be isolated from non- Tfh cells with no labels after *in vitro* differentiation for 5 days. Furthermore, γδ T cells were able to be discriminated from αβ T cells label free using unsupervised machine learning (B).

Conclusions

Here we report on the use of a novel, AI-based cytometry platform to characterize and isolate T cells for C&GT applications. The approach enables label-free isolation of target T cell subsets with defined phenotypic profiles in native states and has practical applications for investigators in basic life science and cell therapy R&D.