# Label-free morphological profiling and isolation of immune cell subsets using VisionSort, a novel, AI-based flow cytometry platform

THINKCYTE

204

Keisuke Wagatsuma[1], Hiroko Nomaru[1], Kazuki Teranishi[2], Satoru Akai[1], Yuri An[1], Kaoru Komoriya[1], Yoko Kawamura[1], Asako Tsubouchi[1], PJ Chana[2], Sadao Ota[1]

[1]ThinkCyte KK, Tokyo, Japan | [2]ThinkCyte Inc, Redwood City, CA

## Abstract

Identification, characterization, and minimally invasive isolation of specific populations of human immune cells are critical for understanding and treating disease. Here we present use cases for VisionSort, a novel artificial intelligence (AI)-driven cell characterization and sorting platform, for isolating specific immune cell subsets label-free. We use machine learning based approaches to identify discrete immune cells subsets including activated T-cells, differentiated B-cells, and macrophages with different polarization states label-free with area under the curve (AUC) performance values ranging from to 0.88-0.99.

## Introduction

Modern cellular immunotherapy approaches demand innovative technologies that can isolate specific immune cell subsets in their native biological/functional states to improve the quality and efficacy of cell therapy drug products. In addition, novel approaches that can assess subtle phenotypic differences in immune cells are needed to advance basic life science research R&D. To this end, technologies that can isolate specific immune cells and immune cell phenotypic subsets without the use of external markers or labels will help investigators and drug developers better understand the native biology of the immune system and develop better immune cell based therapies.

## Methods

Here 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**.

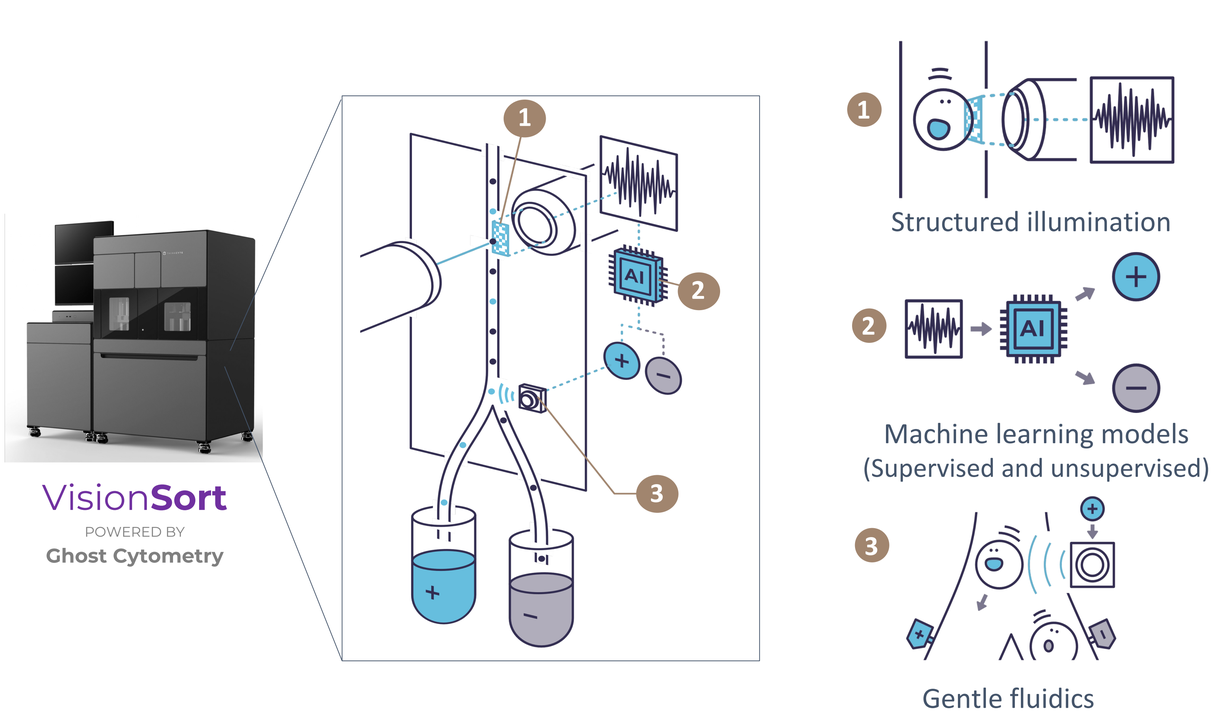


**Figure 1. Principles of the VisionSort platform.** Individual cells pass through a structured illumination (1). Scattered light profiles (reflecting cell morphology) together with fluorescence readouts from individual cells are used together with embedded AI in the instrument (2) to generate machine 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

### Label-free identification of activated T-cells

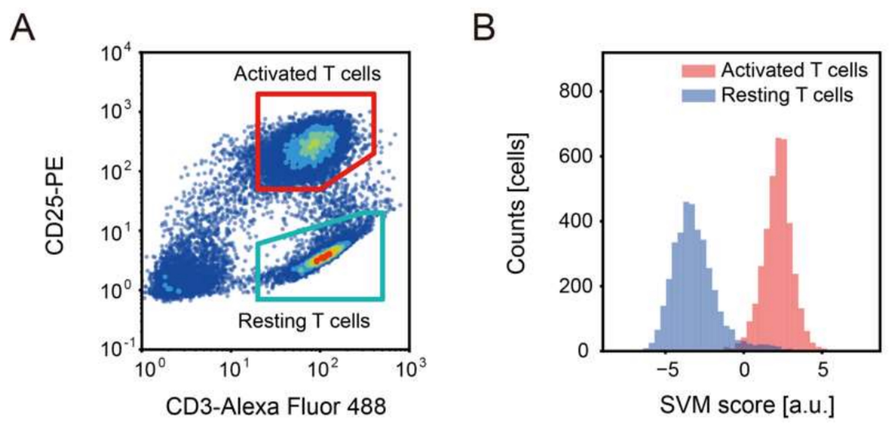


**Figure 2. VisionSort for label-free identification of T cell activation states.** By capturing single-cell digital phenotypes, we characterized human T-cells to generate 'ground truth' functional profiles for activated and non-activated T cells (A). Machine-learning derived classifiers was generated to identify these phenotypic classes in unlabeled T-cell subsets (B). The classifier showed an area under the curve (AUC) performance for differentiating between phenotypically defined T cell populations of 0.990.

### Label-free identification of plasma B-cells

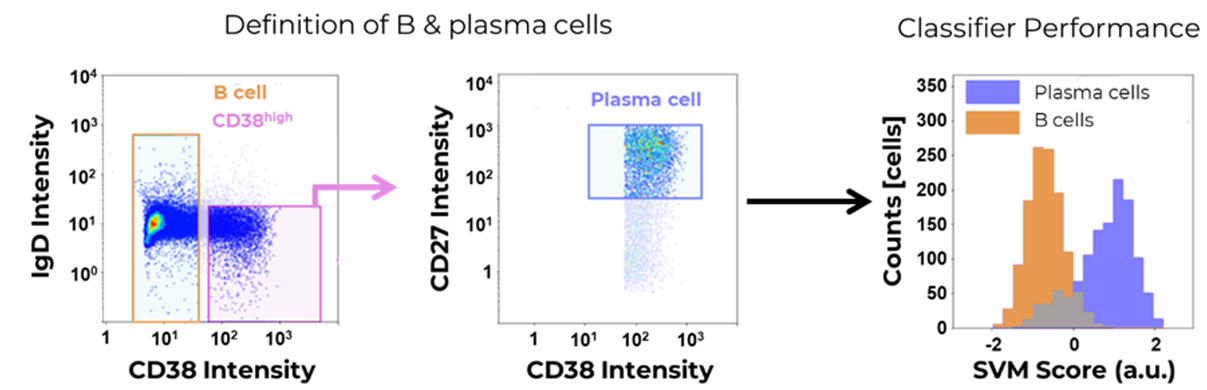


**Figure 3. VisionSort for label-free isolation of plasma B-cells**. Human B cells were cultured under conditions that promoted either B cell activation or plasma cell differentiation. IgD, CD38, and CD27 were used as 'ground truth' markers to define populations of B cells and plasma cells. The VisionSort-derived classifier built using supervised machine learning showed excellent classification of B cells and plasma cells with an AUC score of 0.941.

### Label-free identification of macrophage subsets

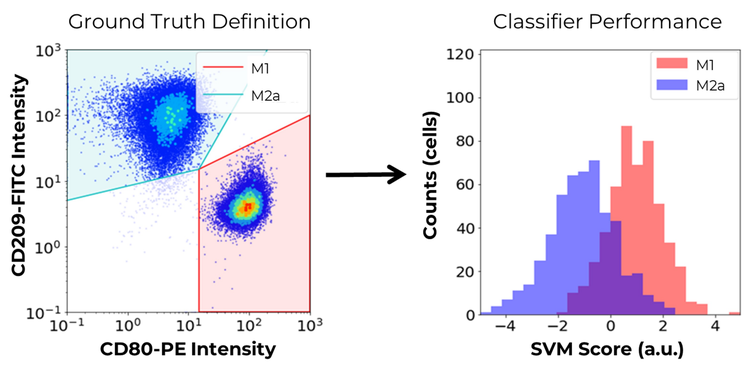


**Figure 4. VisionSort for label-free characterization of macrophage subsets.** From human peripheral blood monocytes, we induced M1 and M2 polarized macrophages in vitro and generated machine learning classifiers. The resulting classifier had high discriminatory power and reproducibility when applied to test samples with an AUC of 0.878 +/- 0.002 (n=6).

## Conclusions

Here we report results on the use of a novel, label-free cytometry platform to characterize and isolate human immune cell subsets using morphological profiling and AI. The approach enables label-free isolation of target immune cell subsets with defined phenotypic profiles in an unperturbed state and has practical applications for investigators in basic life sciences as well as drug developers in small molecule, antibody, and cell therapy R&D.

.