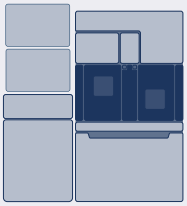
CELL THERAPY

THINKCYTE

Label-free Macrophage Subtyping



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

As monocytes migrate into tissues, they differentiate into macrophages. Upon activation, macrophages can polarize and adopt two very different and biologically opposing phenotypes, termed M1 and M2. M1 macrophages are considered classically activated macrophages that mediate pro-inflammatory responses while M2 macrophages mediate anti-inflammatory responses, both by cytokine secretion. The balance of M1 and/or M2 macrophage polarization is known to be a mediator of physiological responses in tumor progression and as such, the ability to isolate cells in the two polarization states in purified populations is crucial for understanding the dynamics of tumor progression. We show here that VisionSort can be used to isolate pure populations of M1 and M2 macrophages label-free, yielding "untouched" cells ready for downstream R&D applications.

RESULTS

Monocytes were isolated from human PBMCs using anti-CD14 magnetic beads. The cells were cultured in media supplemented with M-CSF (50 ng/ml) for 4 days. On day 4, the media was replaced with supplemented media containing LPS (10 ng/mL) and IFNG (50 ng/mL) to induce M1 differentiation or IL-4 (10 ng/mL) to induce M2a differentiation. Cells were dissociated with a non-enzymatic solution and harvested. Cells stimulated to M1 and M2a differentiation were mixed, stained with fluorescently labeled antibodies to recognize different polarization states (CD80 for M1 and CD209 for M2a) and used to train a supervised machine learning classifier on VisionSort (**Figure 1**). The resulting classifier showed excellent reproducibility with an AUC = 0.878 +/- 0.001 (n=6). Unsupervised analysis correlated with a segregation of the M1 vs. M2a populations (**Figure 2**).

SUMMARY

M1 and M2a macrophages were classified with high accuracy (AUC = 0.878 +/- 0.001) using machine learning. This supervised modeling was confirmed by unsupervised, unbiased UMAP analysis. This study demonstrates the ability of VisionSort to identify and differentiate between M1 from M2a macrophages, yielding an approach that can be used to isolate phenotypically defined macrophages from complex mixtures label-free for downstream R&D.

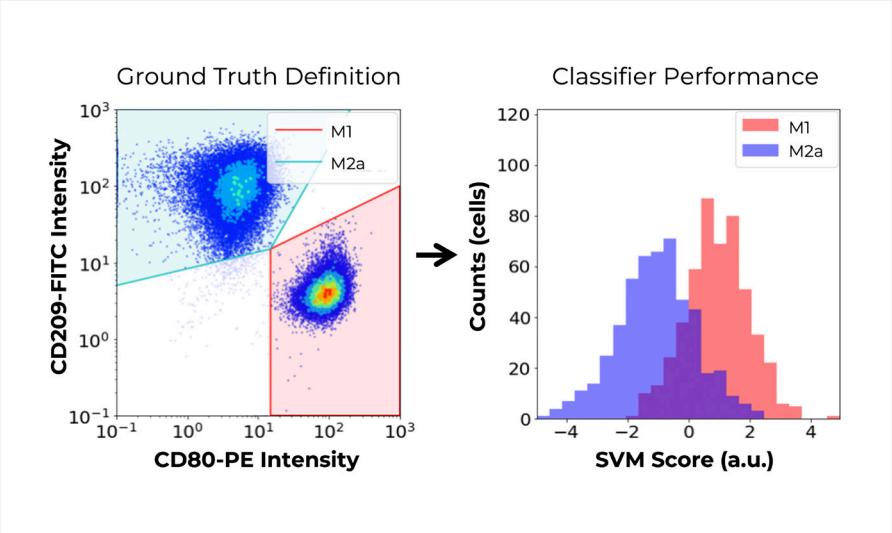


Figure 1. Generation and evaluation of the machine learning classifier for macrophage polarization states. Macrophages were labeled with fluorescently tagged CD80 and CD209 antibodies to define the M1 and M2a subtypes. Morphological profiles from the different ground truth states were used to generate a machine learning classifier on VisionSort, which showed high discriminatory power and reproducibility when applied to test samples with an AUC of 0.878 +/- 0.002 (n=6).

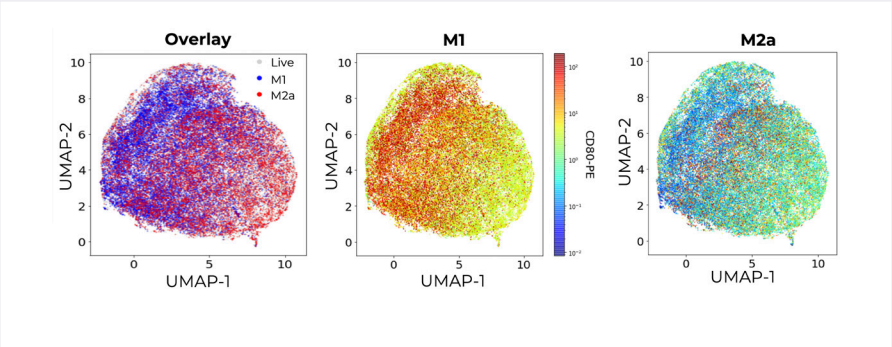


Figure 2. Unbiased evaluation of polarized macrophage populations. Morphological profiles of M1 and M2a macrophages were assessed by unsupervised machine learning on VisionSort and visualized using UMAP. The two cell populations showed distinct morphometric differences. Live: M0 (CD80- CD209-), M1:CD80+ CD209-, M2a: CD80- CD209+.

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