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Characterization and label-free isolation of phenotypically defined human immune cells using Ghost Cytometry, a novel AI-powered high-content flow cytometry technology

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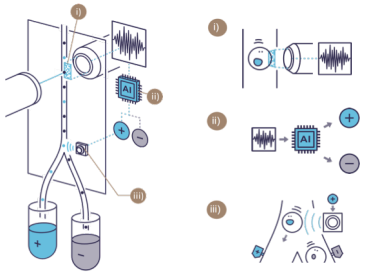
Abstract

Identification and isolation of specific human immune cell populations with technologies that are minimally disruptive to the native biological states are critical requirements in modern cellular immunotherapy approaches. Such technologies can enable the improvement of product quality as well as clinical efficacy of cell therapy drug products, including both autologous and allogeneic cellular therapeutics. Here we applied Ghost Cytometry (GC), a novel method utilizing proprietary optics designs in combination with artificial intelligence to generate high content cellular structural information and enable analysis and sorting of cells in flow without the use of molecular labels (i.e., antibodies). We demonstrate GC's application in characterizing human pan T-cells, where the system was able to identify (i) early apoptosis, (ii) glycolysis level, (iii) exhaustion state, and (iv) activation state with high accuracy in a label-free manner. These results described here highlight a new promising approach for isolation of functionally distinct cells, untouched by external labels, for investigators and drug developers in immunology and immunotherapy research and development.

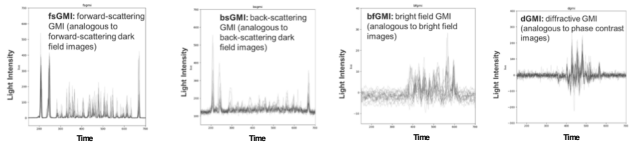
Ghost Cytometry

Ghost Cytometry: Design and concept



- Single cell morphological information is collected as cells pass through a structured illumination and recorded as temporal waveforms (Ghost Motion Image (GMI) signals)
- AI models analyze GMI signals directly for high-speed analysis and sorting
- Target cells are gently collected using fluid pressure

GMI Signals:
Label-free high-content data from scattered light profiles

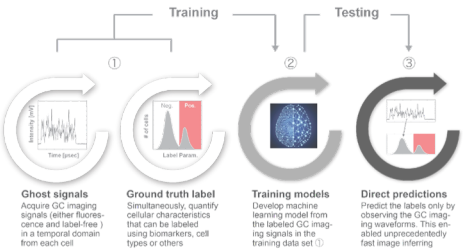


GMI signals are high content and contain more information about a cell's structural morphology than conventional scattered light signals, such as forward and side scatter (FSC/SSC). The collection of GMI signals from a given cell represents its 'cellular fingerprint'.

S. Ota et al., Science 2018. | M. Ugawa et al., eLife 2021

Label-Free T-Cell Analytical Workflow

Machine Learning:
Classifier training and sample analysis workflow



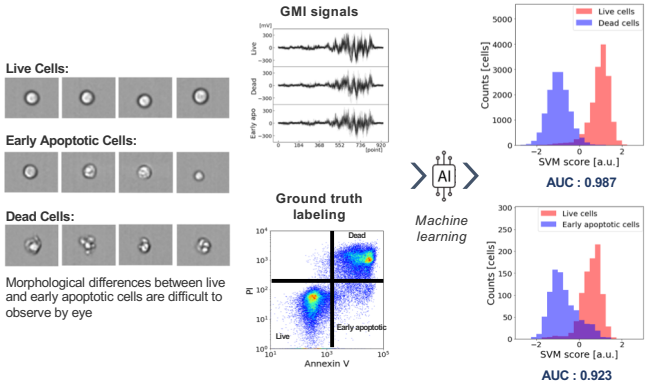
- Ground truth labeling using known markers for phenotypes of interest for initial classifier training. AI learns to map the GMI-based cellular fingerprints to the target phenotype
- Subsequent runs and analysis of samples are conducted completely label-free by applying the developed classifier

Characterizing human pan T-cells with representative phenotypes

Target application	Ground truth labels	Classification
Viability	Annexin V / PI	Live vs. Dead / Apoptotic
Glycolysis	2-NBDG uptake	Low vs. High Glycolytic
Exhaustion	PD1 / LAG3 expression	Exhausted vs. Non-exhausted
Activation	CD3 / CD25 expression	Activated vs. Resting

Results

T-cell viability



Interpretation of classification results

Support Vector Machine Score (SVM score) : A score given to each cell which represents the confidence in (or certainty of) the prediction by a binary classifier trained with an SVM.

Area Under the ROC Curve (AUC): A value used to compare the classification ability of machine learning models. A score closer to 1.0 represent better classification performance.

Results

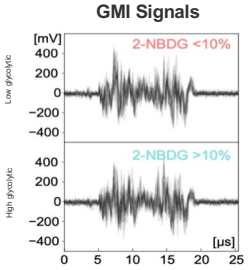
T-cell glycolysis

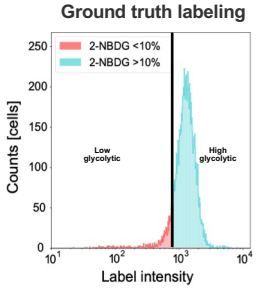
2-NBDG is a fluorescent glucose analog that has been used to monitor glucose uptake in live cells. Low glycolytic cells have less metabolic demands thus lower 2-NBDG uptake.

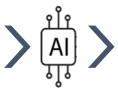
Low Glycolytic Cells:

High Glycolytic Cells:

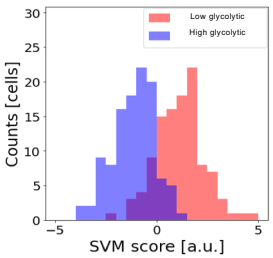
Pan-T cells were cultured with 2-NBDG and incubated for 15 mins to observe uptake







Machine learning



AUC : 0.935

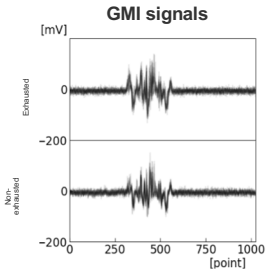
T-cell exhaustion

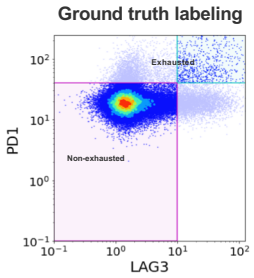
PD1 and **LAG3** are commonly accepted markers for T-cell exhaustion. Here we define double positive in PD1 and LAG3 as exhausted cells, and double negative as non-exhausted cells.

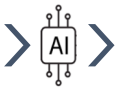
Exhausted Cells:

Non-exhausted Cells:

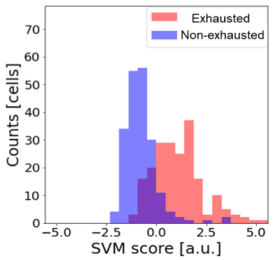
Pan-T cells stimulated with CD3 / CD28 Dynabeads and 25 U/mL of IL-2 for 3 days, then cultured in IL-2 for 11 days







Machine learning



AUC : 0.916

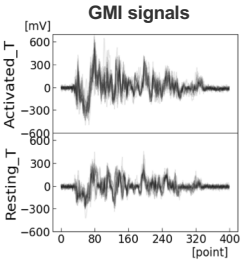
T-cell activation

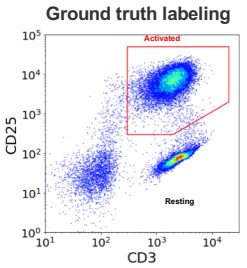
CD3 and **CD25** double positive cells were defined as activated T-cells, while cells expressing CD3 positive and CD25 negative were defined as resting T-cells.

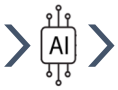
Activated Cells:

Resting Cells:

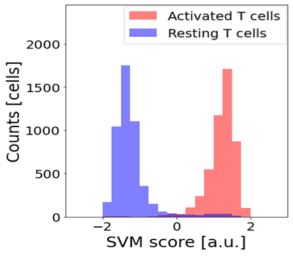
*PBMCs were stimulated with CD3 and CD28 for 5 days, then labeled with **CD25** as the ground truth label for activation.*







Machine learning



AUC : 0.988

Conclusions

- In this study, we demonstrate the use of Ghost Cytometry for label-free analysis of human pan-T cells in characterizing cell health
- Combined with sorting capabilities, Ghost Cytometry can enable researchers access to truly untouched T-cells, avoiding unwanted stimulation or activation by antibody-based labels
- Practical applications include development of better cell-based immunotherapies, discovery of new drug targets in immunotherapy, and automated or semi-automated QC in cell therapy manufacturing.

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