# A Label-free Method to Determine the Glycolytic State of T cells for CAR T Generation

THINKCYTE

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## Abstract

It has become increasingly clear that glycolysis state is an important consideration for the production of T cells into genetically redirected T Cells. Studies indicate that T cells with lower glycolytic activity yielded better anti-tumor outcomes and that current methods tend to drive cells toward terminal differentiation and senescence[i]. Standard FACS with cell sorting required staining the cells with fluorescent analogues of glucose such as 2-NBDG which render the cells not suitable for downstream culture. Thus, the need for a label-free method of isolating T cells with a low glycolysis state to ensure better genetically redirected T cells. Ghost Cytometry with its label-free and AI technology offers a method to ensure the sorting of T cells with low glycolytic activity and suitable for downstream culture and genetic redirection.

[i]Sukumar, M et al, Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest.* 2013; 123 (10) 4479. https://doi.org/10.1172/JCI69589

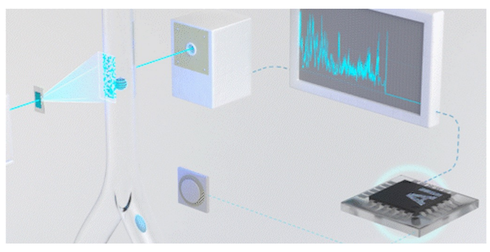
## Introduction

As the field of T cell genetic manipulation expands, the need to monitor cellular health has become an important consideration. The need for a sorting technique that can accurately identify and sort cells with low glycolytic activity without effecting their cellular activity. A standard sorting based on glycolysis would require fluorescent analogues of glucose that would render the cells unusable for downstream applications such as culture or introduction to animal models. We show here that Ghost Cytometry provides a reliable sorting of CAR T cells that have low glycolytic activity, without the need for fluorescent labels, providing a source of T cells that are available for downstream applications.

## Methods

Ghost Cytometry ground truth label training was accomplished by staining the T cells with a fluorescently labeled glucose analog (2-NBDG) and using the lowest 20% vs. the highest 20% of 2-NBDG labeling. This yielded a very high GC Score of 0.986 and precision scores of 0.94 for both low and high glycolytic activity.

VisionSort
POWERED BY
Ghost Cytometry



### Label free Ghost Motion Imaging Signals

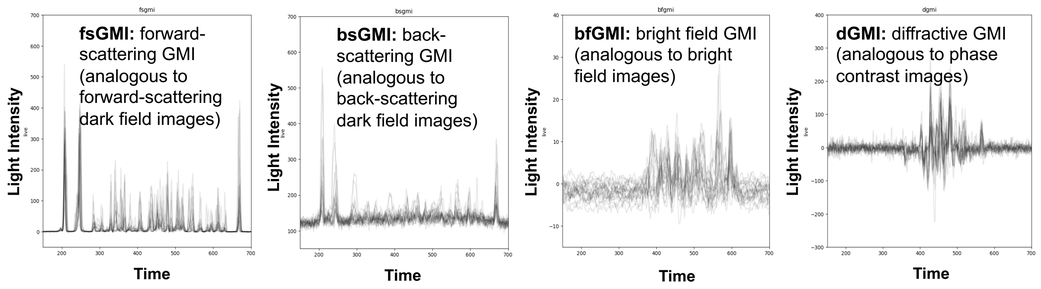



**Figure 1: ThinkCyte® ImageSort™ – Combining Ghost Cytometry with Traditional Fluorescence Cytometry.** ImageSort™ utilizes standard, fluorescent flow cytometry with ThinkCyte® Ghost Cytometry. Ghost Cytometry utilizes structured illumination where the laser lite is diffused through a pinhole structure to reduce the laser intensity and increase the signal to noise ratio. The signals are read by four modalities: forward scatter, back scatter, bright field and diffractive Ghost Motion Imaging (GMI) to create waveforms for each mode. The artificial intelligence interprets the waveforms and uses specific peaks that are predictive of cell populations. Two general modes can be used in Ghost Cytometry: Supervised learning where cells of interest are defined by existing fluorescently labeled biomarkers for Ground Truth Labels

## Results

### Classification of T cells with Low and High Glycolysis activity

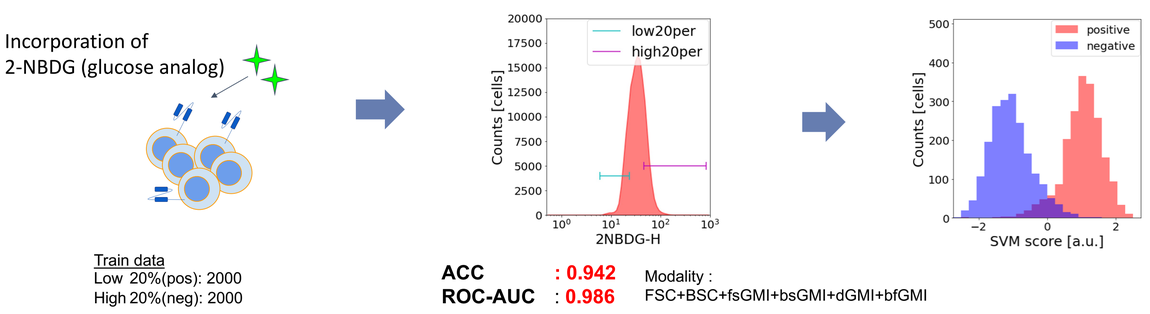
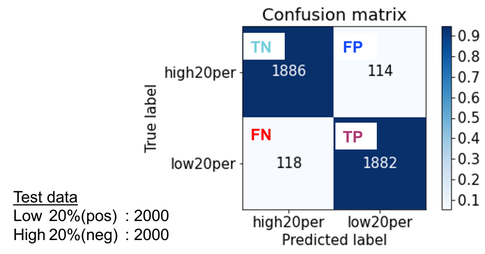


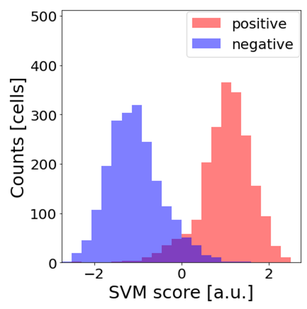

**Figure 2: Ghost Cytometry Ground Truth Label Training Using 2-NBDG Staining Created a Strong AI Model to Sort Cells by their Glycolytic Profile.** Cells were stained with 2-NBDG and assayed by ImageSort® fluorescent detection. The low and high 20% 2-NBDG were gated to define the Ghost Cytometry groups. The separation was significant with a ROC-AUC value of 0.986 for all T cells. The training set consisted of 2000 cells for both the low and high glycolysis (low and high 2-NBDG staining).

## RESULTS

### Ghost Cytometry Prediction Result




| class | precision | recall | f1-score |
|---|---|---|---|
| high20per | 0.941 | 0.943 | 0.942 |
| low20per | 0.943 | 0.941 | 0.942 |




**ACC : 0.942**
**ROC-AUC : 0.986**

Modality :
FSC+BSC+fsGMI+bsGMI+dGMI+bfGMI

**Figure 3: Classification Results of Low Glycolytic vs. High Glycolytic CAR-T Cells Validated the Supervised Ground Truth Modeling.** After Ghost Cytometry was trained, the same set of cells were reintroduced into the GC instrument to determine how well the predicted label would match the true label; the Ghost Cytometry AI model is validated.

### Microscopic Confirmation of Cellular Difference due to Glycolysis

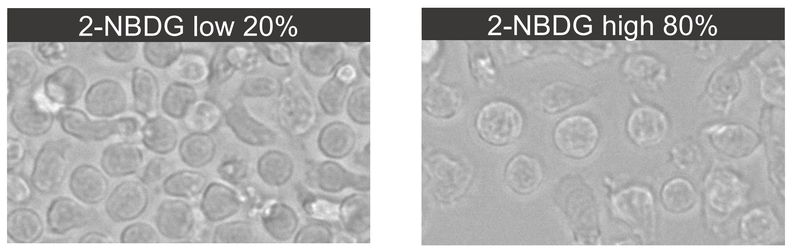
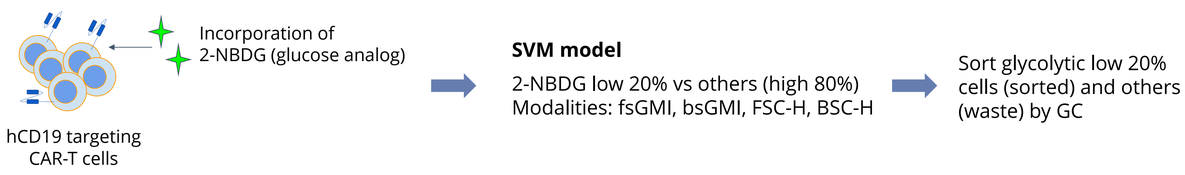



**Figure 4: Microscopic Morphological Characteristic Confirmation of CAR T cells with Different Glycolysis Levels.** Cells with low 2-NBDG staining showed very different cell shape indicating significant cytoskeletal change corresponding with differential glycolytic activity. Ghost Cytometry was able to determine cells with low and high glycolytic activity with high accuracy. Image Sort can separate one defined cell population at a time. In this case the Low 20% glycolytic activity were sorted from the remaining 80% of the cells. The cells can then be resorted to attain additional cell populations.

### Genomic Confirmation of Ghost Cytometry Sorted Cells




PKM – Pyruvate Kinase M – a glycolysis related gene
TCF7 - Transcription Factor 7 – high expression associated with naïve or early memory T cells (which correlates with low glycolysis)
PDCD1 – Programmed Cell Death Protein 1 – High expression is associated with activated or exhausted T cells (Low expression correlates with low glycolysis)

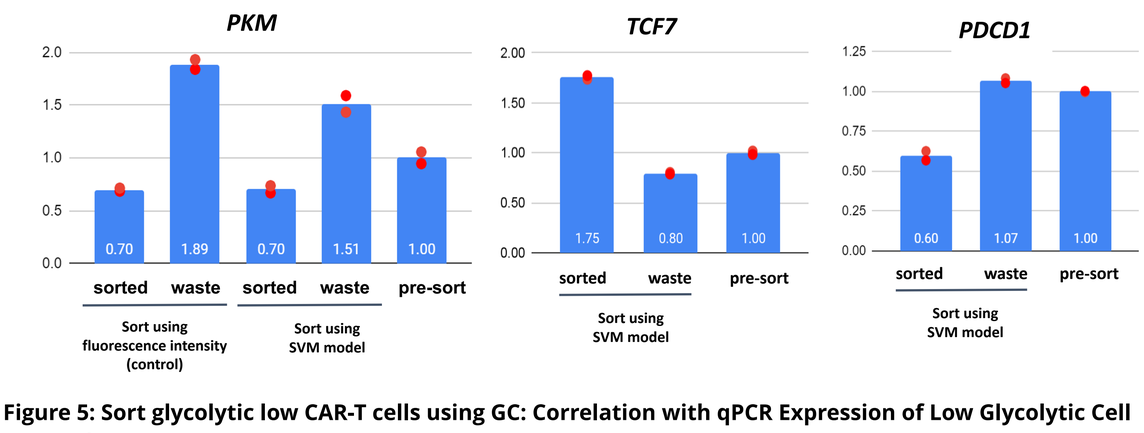



**Figure 5: Sort glycolytic low CAR-T cells using GC: Correlation with qPCR Expression of Low Glycolytic Cell Expression**

## Conclusions

1. Low glycolytic CAR T cells could be identified by Ghost Cytometry (GC)
2. Low glycolytic CAR T cells identified by GC correlated very well with standard flow cytometry techniques using 2-NBDG
3. Low Glycolysis classification also correlated with microscopy morphology data
4. Low Glycolysis classification correlated with qPCR data of PKM and TCF7 and PDCD1 markers correlated with low glycolytic activity.
5. Label – free Ghost Cytometry is an effective technique to isolate low glycolytic CAR T cells for downstream uses