THINKCYTE

APPLICATION NOTE

Intracellular Phenotyping in Flow with Fluorescence Ghost Cytometry

INTRODUCTION

Many cellular processes and functions involve phenotypic changes or biomolecular interactions at the intracellular level. Researchers can exploit knowledge of these phenotypic changes to better elucidate basic cellular biology, profile disease pathology, or identify new drug targets. Classically, cellular phenotypes have been assessed using high-resolution, image-based microscopy approaches. However, microscopy-based approaches are slow, manual, and time consuming. Recently, phenotypic assessment using flow cytometry has been developed, offering substantially higher throughput. However, conventional flow cytometers only collect data on the average intensity of light signals and can not deeply analyze intracellular phenotypes with a high degree of spatial resolution. In addition, imaging flow cytometers are unable to sort cells based on intracellular phenotypes at high speed. We have developed a fluorescence-based ghost cytometry (FL-GC) technique that enables both rapid analysis and sorting of cells based on intracellular phenotypes with high spatial resolution. As proof-of-concept, we demonstrated FL-GC's ability to detect nuclear translocation, organelle localization, and protein-protein interaction within single cells.

RESULTS

The cellular phenotype of NFkB nuclear translocation was induced in THP-1 cells by LPS stimulation. Cells were fixed, stained with NF-kB p65 (D14E12) XP, and analyzed via FL-GC (**Figure 1**). Comparing LPS stimulated and non-stimulated populations by supervised machine learning, FL-GC was able to detect the two distinct phenotypes with an outstanding classifier accuracy AUC score of 0.995.

Detection of target molecules in either the mitochondria or lysosome was used to demonstrate FL-GC's ability to analyze organelle localization phenotypes (**Figure 2**). Live HEK293T cells were cultured with MitoTracker and LysoTracker to stain the mitochondria and lysosomes, respectively, as the ground truth labels. FL-GC was able to achieve an outstanding classifier accuracy to discriminate between these populations, with an AUC score of 0.999.

The protein-protein interaction phenotype was assessed by simulating a fluoppi cell line, p53/MDM2 CHO-K1, which forms p53/MDM2 puncta within the cells when stimulated with Nutlin-3a (**Figure 3**). Fixed cells expressing both phenotypes were used as ground truth labels to train a classifier via FL-GC. The classifier demonstrated outstanding accuracy with an AUC score of 0.958.

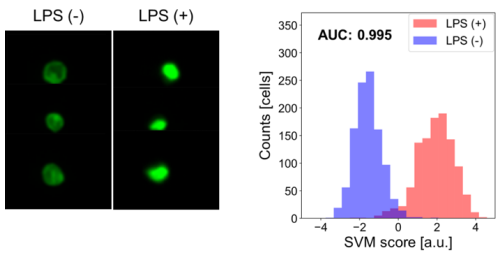

Figure 1. LPS stimulation of THP-1 cells induced the nuclear translocation of NF-kB as stained and visualized by fluorescence microscopy. Cells were fixed and analyzed with FL-GC for training and testing a classifier, which demonstrated outstanding accuracy (AUC of 0.995).

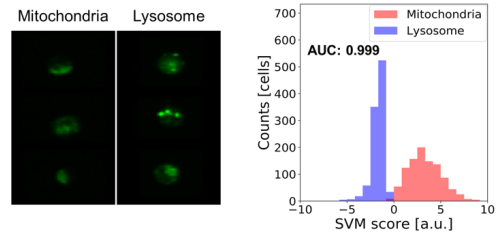

Figure 2. Live HEK293T cells were cultured with MitoTracker and LysoTracker to stain the mitochondria and lysosomes, respectively. Mixed populations of cells were used to train and test a classifier via FL-GC. The classifier showed outstanding performance with an AUC of 0.999.

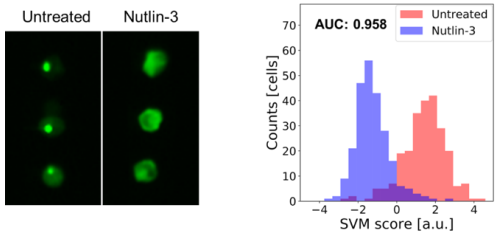

Figure 3. Nutlin-3 was used to induce the intracellular p53/MDM2 protein-protein interaction in a fluoppi p53/MDM2 CHO-K1 cell line. The phenotype was confirmed using fluorescence microscopy. Mixed populations were used to train and test a classifier by FL-GC. The classifier achieved outstanding performance with an AUC of 0.958.

SUMMARY

Intracellular phenotypes such as nuclear translocation, organelle localization, and protein-protein interaction were successfully detected by FL-GC. The approach was able to develop AI-based classifiers with high accuracy. This proof-of-concept has practical utility in drug discovery and disease profiling, where a wide range of cellular phenotypic changes are profiled. The ability to detect these phenotypes with spatial resolution in a flow-based system represents advantages over existing technologies and has the potential to accelerate the advancement of early drug discovery.

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