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Monitoring Stem Cell Differentiation Using Raman Spectroscopy, Microfluidics, and Machine Learning

Induced pluripotent stem cells (iPSCs) hold enormous promises for many biomedical applications, such as regenerative medicine, drug testing, and disease modeling. One application is the production of natural killer (NK) cells, which can be derived from iPSCs. NK cells are one of the most promising and potent immunotherapeutic approaches for cancer. Clinical applications of stem cells require the identification of characteristics at the single-cell level and continuous monitoring during differentiation to optimize manufacturing and discovery processes for stem cell-based therapies. Various microscopy techniques can be used to resolve the structural and functional characteristics of a cell, usually by targeting a specific subcellular component or function using fluorescent labels. However, specific labels might not capture important heterogeneity in the cell population, e.g. a population of NK cells identified by a few fluorescent labels might consist of more subgroups, initially not detected by the fluorescent markers. Raman spectroscopy offers a label-free, non-invasive alternative that, when combined with unsupervised Machine Learning (ML) methods like autoencoder-based spectral unmixing and manifold learning, has the potential to uncover heterogeneity not captured by Fluorescence-Activated Cell Sorting (FACS) [1]. In this study, we demonstrate the ability of Raman spectroscopy, integrated with ML analysis, to distinguish NK cell subpopulations at different stages of expansion and activation, under various culture conditions. These findings are supported by FACS reference data. Additionally, we explore the implementation of Raman-based analysis within a microfluidic platform, enabling cell sorting and offline validation through established techniques such as proteomics and RNA sequencing.

References

[1] J. Geng, W. Zhang, C. Chen, H. Zhang, A. Zhou, and Y. Huang., Analytical Chemistry, 2021, 93(30): 10453-10461.

Figures

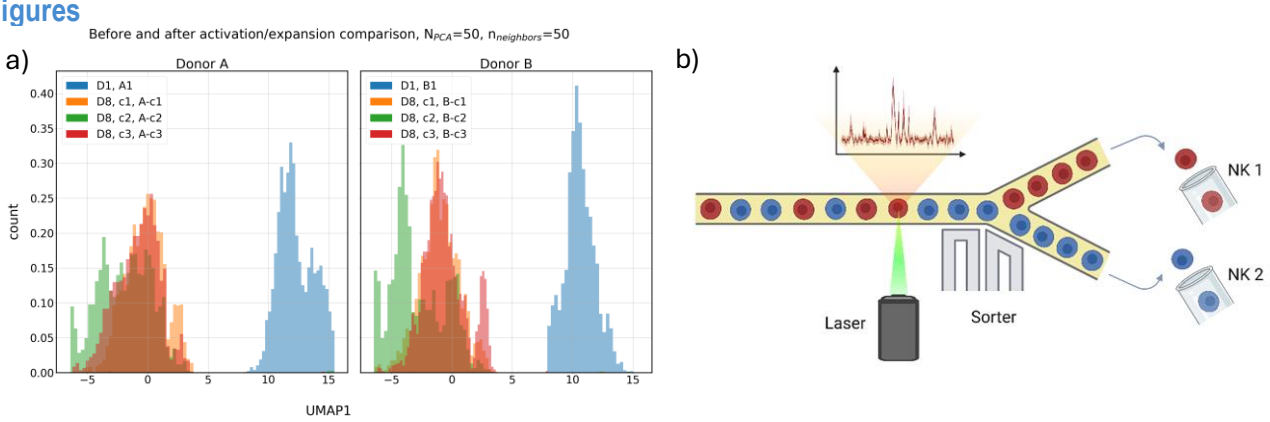

Figure 1: a) Distribution of Raman spectra from single NK cells before and after activation by cytokines (C1: IL-2 + IL-15, C2: IL-12 + IL-15 + IL-18, C3: IL-15 + IL-21), visualized by UMAP embedding. b) Capillary-based Raman activated cell sorting platform.