AUTOFLUORESCENCE LIFETIME IMAGING TO MONITOR IMMUNE CELL METABOLISM AND FUNCTION

Melissa C. Skala, Morgridge Institute for Research and University of Wisconsin - Madison
mcskala@wisc.edu
Rebecca L. Schmitz, Morgridge Institute for Research
Kelsey E. Tweed, Morgridge Institute for Research
Kayvan Samimi, Morgridge Institute for Research
Jeremiah Riendeau, Morgridge Institute for Research
Emmanuel Contreras Guzman, Morgridge Institute for Research
Matthew H. Forsberg, University of Wisconsin - Madison
Ankita Shahi, University of Wisconsin - Madison
Jose M. Ayuso, University of Wisconsin - Madison
Christian M. Capitini, University of Wisconsin - Madison

Key Words: FLIM, immunology, metabolism, single cell analysis.

New non-destructive tools are needed to reliably assess lymphocyte function for immune profiling and adoptive cell therapy. Optical metabolic imaging (OMI) is a label-free method that measures the autofluorescence intensity and lifetime of metabolic cofactors NAD(P)H and FAD to quantify metabolism at a single-cell level. Here, we demonstrate that OMI can quantify metabolic changes in human immune cells from peripheral blood. This approach is attractive because it does not require cell surface labels or transfection, enabling rapid assessment of single cell metabolism. Newly trained neural networks were used to automatically segment single cells for analysis of heterogeneity within and between patients. This single cell analysis found that the NAD(P)H mean fluorescence lifetime increased in NK cells compared to T cells, and further increased in B cells compared to NK cells. Random forest models based on OMI classified lymphocytes according to subtype (B, NK, T cell) with 97.8% accuracy, and according to activation state (quiescent or activated) and subtype (B, NK, T cell) with 90.0% accuracy. Our results show that autofluorescence lifetime imaging can accurately assess lymphocyte activation and subtype in a label-free, non-destructive manner. Overall, this approach is attractive for both basic research and patient management in cancer and immunology.

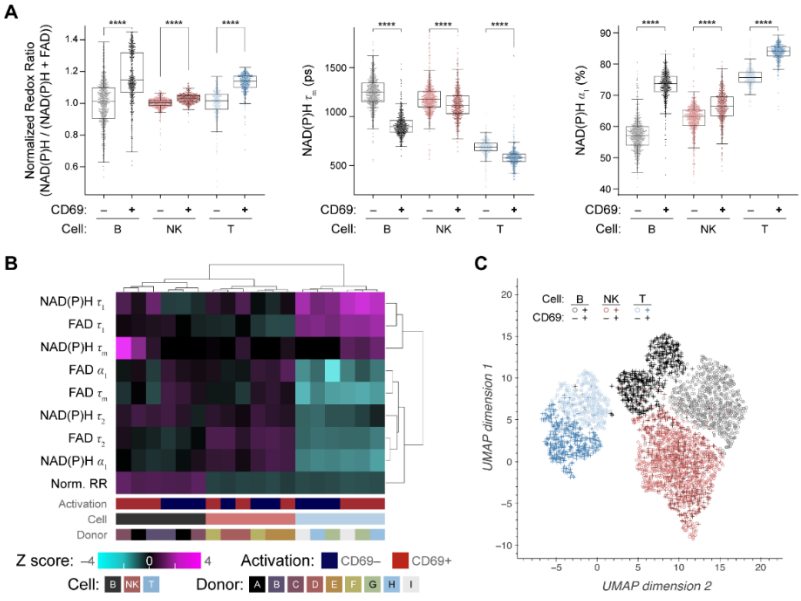

*Figure 1: OMI parameters for quiescent and activated B cells, NK cells, and T cells. (A) Box-and-whisker plots of key OMI variables display consistent changes with activation across T cells, B cells, and NK cells. (B) Heatmap displaying hierarchical clustering of groups of activated or quiescent cells by lymphocyte subtype, human blood donor, and activation status, calculated from the z-scores of OMI variables. (C) UMAP of single-cell OMI data displays distinct clusters of lymphocytes based on lymphocyte subtype and activation status. 749 CD69- control B cells, 461 CD69+ activated B cells, 667 CD69- control NK cells, 554 CD69+ activated NK cells, 331 CD69- control T cells, 365 CD69+ activated T cells. **** P < 0.0001, Kruskal-Wallis with post-hoc comparisons. ns = not significant. (Schmitz et al. BioRxiv 2023)*