Automated Analysis of Flow Cytometry Data to Reduce Inter-Lab Variation in the Detection of Major Histocompatibility Complex Multimer-Binding T Cells - DTU Orbit (07/04/2019)

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Manual analysis of flow cytometry data and subjective gate-border decisions taken by individuals continue to be a source of variation in the assessment of antigen-specific T cells when comparing data across laboratories, and also over time in individual labs. Therefore, strategies to provide automated analysis of major histocompatibility complex (MHC) multimer-binding T cells represent an attractive solution to decrease subjectivity and technical variation. The challenge of using an automated analysis approach is that MHC multimer-binding T cell populations are often rare and therefore difficult to detect. We used a highly heterogeneous dataset from a recent MHC multimer proficiency panel to assess if MHC multimer-binding CD8(+) T cells could be analyzed with computational solutions currently available, and if such analyses would reduce the technical variation across different laboratories. We used three different methods, FLOW Clustering without K (FLOCK), Scalable Weighted Iterative Flow-clustering Technique (SWIFT), and ReFlow to analyze flow cytometry data files from 28 laboratories. Each laboratory screened for antigen-responsive T cell populations with frequency ranging from 0.01 to 1.5% of lymphocytes within samples from two donors. Experience from this analysis shows that all three programs can be used for the identification of high to intermediate frequency of MHC multimer-binding T cell populations, with results very similar to that of manual gating. For the less frequent populations (