Automated and Standardized Clustering of Flow Cytometry Data

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Flow cytometry (FCM) offers a rapid quantification of multidimensional characteristics for millions of cells and is widely used in health research and in treatment for a variety of tasks, e.g. the diagnosis and monitoring of rheumatic patients.

During traditional analysis these data are reduced using a manual or semiautomated process of gating successively on 2-D projections of the data. This process requires an immoderate amount of time and is error-prone and non-reproducible. Since the number of measured parameter in modern devices is still increasing the manual analysis step is the most limiting aspect of the technology and embarrasses a high throughput analysis in FCM.

Recently several approaches for automated gating were introduced but so far no full automated feature extraction software tool for FCM data is available. We developed an unbiased unsupervised algorithm based on Expectation Maximization (EM)-iteration for this task which is applicable even for large FCM data sets (10 parameter and more than 1 million events). Staining whole blood simultaneously in a multiparametric cytometric profiling assay, analysis with this algorithm correctly identified and distinguished all major populations including neutrophils (CD16+), T-cells and subpopulations (CD3+, CD4+, CD8+), monocytes (CD14+), B-cells (CD19+), and NK-cells (CD56+) and thus was comparable to the so far “gold standard” of manual evaluation by an expert. In addition, the new technology is able to detect subclusters and to characterize so far neglected smaller populations based on the new parameters generated to identify and characterize the cytometric clusters.

The algorithm is currently tested and further developed for comparative analysis between healthy donors and patients suffering from chronic inflammatory diseases like rheumatoid arthritis, ankylosing spondylitis or systemic lupus erythematosus.