High-throughput multiparametric flow cytometry – how does the new hardware impact our interaction with data?

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Abstract

High-throughput flow cytometry (HT-FC) is an emerging technology of single-cell analysis pairing the high-content capability of traditional polychromatic FC with automated sample handling and processing systems. HT-FC is able to process samples arranged in a complex multifactorial experimental format, which may include a series of samples exposed to various compounds in multiple concentrations, , at different temperatures, and/or at certain time points. Since every HT-FC experiment produces a vast amount of information, it is imperative to link the HT-FC system with an automated data acquisition and processing pipeline, which can facilitate fast design and implementation of screening formats and can automatically process and mine the collected FC data. The multifactorial datasets collected by HT-FC are difficult to visualize and/or understand in the context of traditional FC data analysis approaches. The typical low-throughput FC data analysis is performed in an exploratory fashion. This involves orthogonalization of the fluorescence-related features and subsequent interactions with the data sets, employing simple two-dimensional visualization techniques in order to produce a cascading series of density plots. The researcher (data analyst) is expected to possess expert knowledge that permits him to streamline the visualization and to find relevant data projections and feature combinations that lead to identification of expected populations and allow for semi-quantitative assessment of changes. In contrast to traditional FC, but similar to image-based high-throughput screening, the individual analytical steps in HT-FC systems are not expected to be human-readable or interpretable. However, they are expected to be reproducible, robust, and amenable to high-dimensional data formats. Therefore, the automated analytical procedure conceived for HT-FC may be designed without the constraints of mimicking the human-guided discovery process. This presentation will discuss the contrasting philosophies of traditional FC and automated HT-FC. It will also briefly summarize our attempts to build a HT-FC acquisition and data-processing pipeline capable of performing multifactorial assays, extracting features from FC datasets, computing secondary parameters based on observer-independent statistics, and producing a concise output summarizing the screen information. This described system utilizing Beckman Coulter cytometers and laboratory robots coupled with a HyperCyt autosampler is capable of collecting and analyzing data from a 384-well-plate in less than 10 minutes.

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Keywords: High, throughput, multiparametric flow cytometry, hardware, data analysis