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High Throughput High Dimensional Multi-parametric Analysis of the Immune System

Status	Current
Competition	Development of New Technologies Competition
Sector	Development of New Technologies
Genome Centre	Genome Quebec
Project Leader	Raffick-Pierre Sékaly & Ryan Brinkman

Project Description

Flow cytometry (FCM) is a technique for counting, examining, and sorting microscopic particles, usually cells, suspended in a stream of fluid. As the cells flow through an optical/electronic detection apparatus multiparametric measurements of the cells' physical and/or chemical characteristics are made by a series of one or more light sources (usually lasers) and detectors. Each suspended particle passing through the beam scatters the light and fluorescent chemicals found in the particle, or attached to the particle during a labeling process designed to target specific proteins. This combination of scattered and fluorescent light is picked up by the detectors, and by analyzing fluctuations in brightness at each detector it is then possible to extrapolate various types of information about the physical (e.g., size and shape) and chemical composition (e.g., relative amount of protein present) of each individual particle. FCM is proteomic technique that can be used for the identification of cell populations and/or for physically sorting and separating the cells. The technology is applied to many fields of science, including molecular biology, pathology, immunology, plant biology and marine biology. In the field of molecular biology it is especially useful when used with fluorescence tagged antibodies. These specific antibodies bind to proteins on or in the target cells and help to give information on specific characteristics of the cells being studied in the cytometer. FCM is integral part of proteomic and genomic technology. It has broad application in medicine (especially in transplantation, hematology, oncology, immunology, chemotherapy, and genetics). In marine biology, the auto-fluorescent properties of photosynthetic plankton can be exploited by flow cytometry in order to characterize abundance and community structure. In protein engineering, flow cytometry is used in conjunction with yeast display and bacterial display to identify cell surface-displayed protein variants with desired properties.

FCM can generate complex datasets both in terms of the number of parameters measured for each individual cell (over 20), as well as the number of individual cells analyzed per experiment (in the millions). As a result, the amount of data that is generated is enormous (gigabytes to terabytes), and the analysis is complex and very time consuming. Unfortunately, the bioinformatic tools available for FCM analysis have lagged behind other platforms like sequencing, arrays mass spectrophotometry etc.

The objective of this project is to create new tools able to take advantage of the high dimensionality of high throughput FCM (HT-FCM) data and to combine the result with genomics and proteomics. The methods proposed here will revolutionize the current basic academic and commercial tools in the scientific community. The integration of HT-FCM, genomics and proteomics data will provide a deeper understanding of specific immune cell behavior. The functional relationships identified will provide new knowledge enabling the elucidation of disease mechanisms, the prediction of therapeutic and vaccine outcome, and the development personalized medicine. To do so, we will create (1) a High Throughput Flow Analysis Application (HT-FAA) to remove the excessive manual labor associated with the current 2-dimensional analysis methods and provide statistically sound, objective analysis methods to take advantage of the multivariate nature of HT-FCM data using true multi-dimensional analysis techniques. We will develop (2) a High Throughput Reporting Application (HT-RA) for creating novel visualizations for HT-FAA processed data and for integrating the results with other functional (genomics, proteomics) data and public scientific annotations, using novel visualizations into multi-dimensional data. Finally, we will define (3) a Patient Immuno Profiling Process (PIPP) by improving experiment reporting and presenting a more complete functional genomic view of cells involved in the immune system.