

Rare Event Detection of Cells Expressing Stem Cell Markers in Normal Lung Tissue Using the VenturiOne™ Analysis Software.

Introduction

Rare-event detection is becoming an increasingly important application of flow cytometry. The data files that are generated can consist of 5 to 14 parameters measured on as many as 10 million events for analysis. This is usually a long drawn out process due to the lack of software designed to analyse large data files. For example, in a recent study to identify stem cell marker-positive cells among rare solid tumour cells, the data set consisted of approximately 50 eight-colour data files of 250,000 to 2.5 million events each. Other rare event applications include detection of foetal cells in maternal blood, dendritic cell biology or even malaria diagnosis to name but a few.

VenturiOne™ analysis software was developed in order to process large data files generated in rare event detection. In the following procedure, VenturiOne™ is used to analyse 2.4 million events.

Method

1. Mince human lung tissue using a Becton Dickinson Medimachine
2. Filter tissue through a 70- μ cell strainer
3. Digest with collagenase
4. Separate on a Ficoll-Hypaque gradient
5. Stain the cell suspension with the following antibodies: CD90-ECD, ABCG2-PC5, CD117-PC7, CD133APC and CD45-APCC7
6. Fix and permeabilize with saponin
7. Stain cells with anti-cytokeratin FITC and anti-p53 PE
8. Immediately before acquisition, add the DNA stain DAPI
9. Perform acquisition of sample using a flow cytometer collecting a total of 2.4 million events.
10. Spectral compensation and data analysis is performed on the list mode data files using VenturiOne™ software.

Results

Analysis of the multi-parameter data is performed in three steps: (1) gating on the populations of interest (classifier populations), (2) detection of outcome parameters on populations of interest and (3) exploring the relationship between classification parameters and outcome parameters through colour eventing (Fig.1).

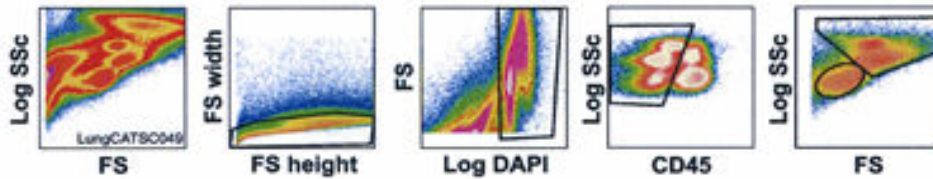


Fig 1. Gating strategy for multi-parameter analysis of stem cell marker-positive cells in disaggregated human lung tissue. Doublet discrimination, DAPI staining, and light scatter properties were used for data cleanup, eliminating cell clusters, hypodiploid cells and subcellular debris. After cleanup, CD45 and side scatter were used to define haematopoietic (CD45+) and nonhaematopoietic populations. Light scatter properties were used to divide the CD45-negative population further into cells with simple versus complex morphology.

The two classifier populations carried forward for analysis of outcomes are CD45-negative cells simple morphology and CD45-negative cells of complex morphology (Fig 2).

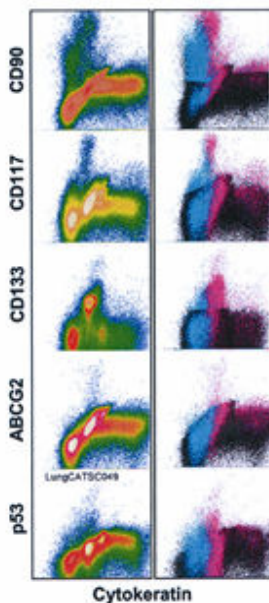


Fig 2. Use of density histograms and colour-evented dot plot to evaluate classifier variables in the context of outcome variables. Candidate stem and progenitor populations are colour-evented cyan and magenta, respectively, with cyan having colour precedence.

Conclusion

VenturiOne™ is a new generation of specialised analytical flow cytometry software that has the advantage of parallel processing, extensive random access memory capacity and is also designed to work with Microsoft's new 64-bit Windows XP and Windows Vista operating systems. Not only does it have the features of conventional data analysis software in which histograms, logical gates and analytic regions are created but it also provides a preview tab that displays all possible one and two parameter histograms. The preview allows you to focus on populations that are defined by

any gate created within the analysis space. Any graph in the preview page can be copied to the analysis page by a simple double-click, where regions and various tools can be applied to the results for further analysis.

Complex analyses of large list mode data files can be performed efficiently, with ease and in a timely manner due to the use of VenturiOne™ analysis software.

Related Publications

Donnenberg and Donnenberg, 2007, Rare-Event Analysis in Flow Cytometry, Clinics in Laboratory Medicine 27:627-652