Insights and Expanding Practical Education in a Core Facility with Brightfield Imaging-Enabled Flow Cytometry

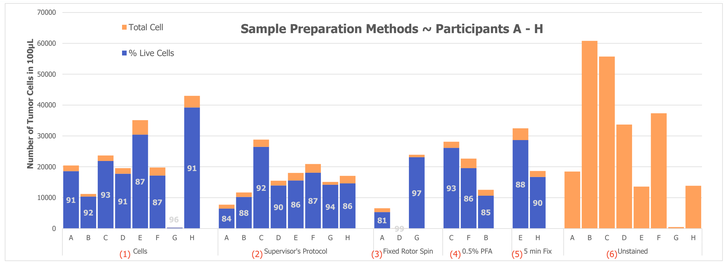
Cora H. Chadick, Priscilla Heijnen, Tanja Konijn, Susanne van der Pol, Carolien Zeelen

Objective

One of the many roles of flow cytometry (FC) core facilities and shared resource laboratories typically occupy is that of educator and trainer to assure that researchers of all levels can quickly comprehend basic principles of the FC method. Those who are new to the world of conventional FC often do not find the interpretation of FC data from a dot plot or histogram to be intuitive and often struggle to find appropriate gain or voltage settings for light scatter parameters. Even some seasoned cytometrists can find it difficult to assess appropriate scatter and coincidence gating from dissociated tissue or other sample types with highly variable scatter properties among events.

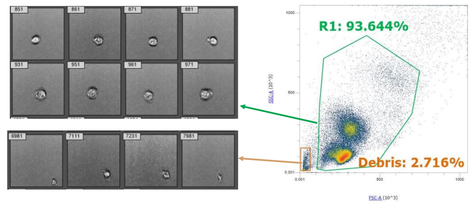
The addition of the **Attune CytPix** from ThermoFisher Scientific to the Microscopy and Cytometry Core Facility has become a key asset in providing quick feedback for the assessment of sample quality, setting scatter parameter sensitivity, and having direct feedback on gating strategies. This is entirely because of the capability of the CytPix which couples brightfield images to fcs data allowing for the display of captured images corresponding to regions on plots and vice versa. This easily enables researchers to instantly and directly assess scatter settings and gating strategies without the time-consuming process of cell sorting followed by inspection at a microscope or the inability to capture the conventional 488nm forward and side scatter information as is the case in most, much slower, imaging flow cytometers for quick troubleshooting at a conventional FC system.

Practical Workshop Results

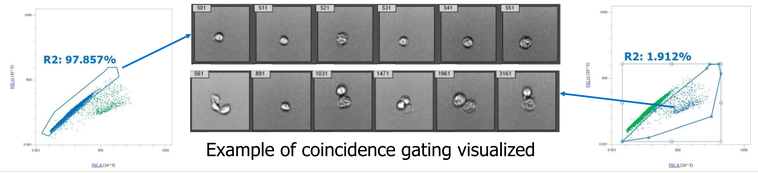


As part of the practical introductory workshop, samples were prepared by student and lab technician participants. Each person was given the same protocol to follow in addition to contrived, non-ideal preparation conditions. Frozen colon cancer cultured cells were stained with fixable viability dye eFluor 780 or remained unstained and were (1,6) fixed in 2% PFA for 15 minutes and spun with a swing bucket rotor or (2) prepared according to their supervisor's standard protocol and (3,4,5) 3 variable non-ideal conditions. Sample recovery and viability is shown per participant and preparation conditions.

Practical Workshop – Fundamental Concepts

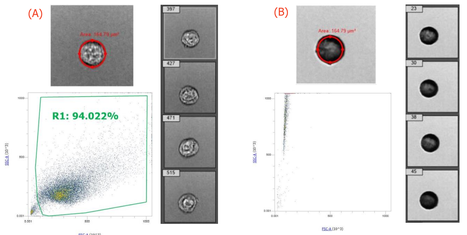


Characterize the separation between cells and debris with the 2 cell types they prepared for the workshop; tumor cell culture and PBMC. Shown: cell vs debris clouds in PBMCs prepared by workshop participants.

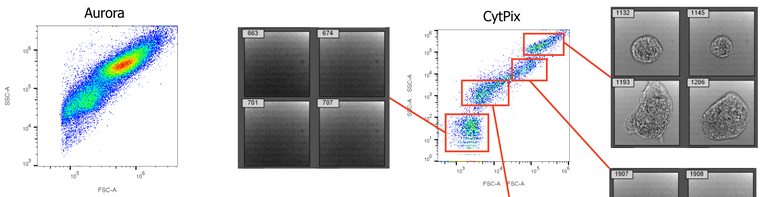


Example of coincidence gating visualized

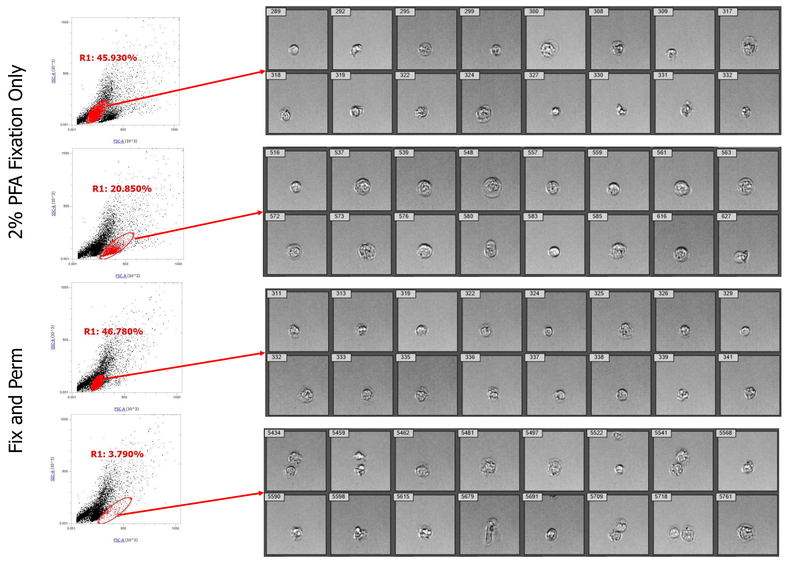
Correct the oversimplified concept that SSC and FSC equate to size and complexity by providing participants an illustration towards a more complete understanding of scatter parameters to be refraction, reflection, diffraction, and absorption/radiation. Shown: (B)polystyrene beads as compared to (A)cells of a similar average size both acquired with the same detector voltages.



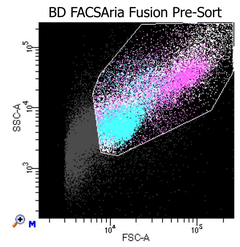
Insights and Troubleshooting Approaches



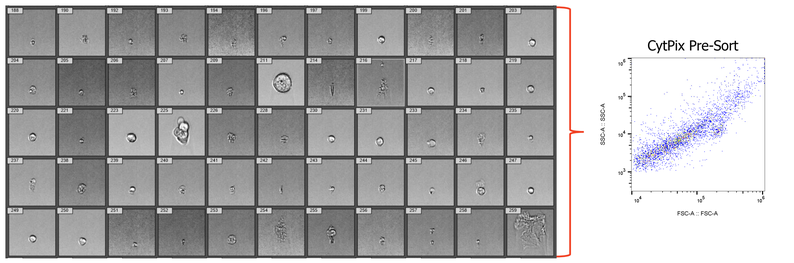
Fibroblast culture FSC v SSC gate assessment on the CytPix to quickly validate single-cells in the high-scatter cloud found at a Cytek Aurora spectral analyzer. *Note, the primary SSC is off of 405nm laser at the Aurora.

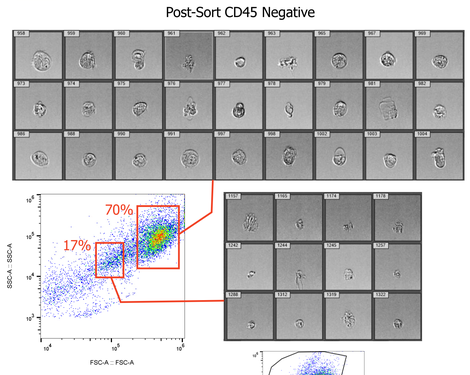


A researcher wanted insights into their cell loss during fixation and permeabilization during PBMC sample preparation. After observing images of their cells associated with scatter, they looked more deeply into their protocol and discovered they were using 1% Saponin rather than 0.1%.

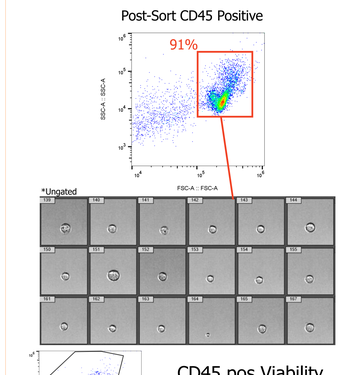


Dissociated head and neck tumor pre- and post-sort top level gate assessment was viewed on the CytPix for cells to be loaded onto an emulsion single-cell isolation and bead-barcoding system which was not capturing adequate mRNA for library construction and sequencing. The usual workflow quantified cell count and viability based on an electrical impedance device and post-sort samples were re-run on the Aria Fusion. Comparison of CytPix images support the other 2 validation methods indicating a majority of intact cells after the sorting process.

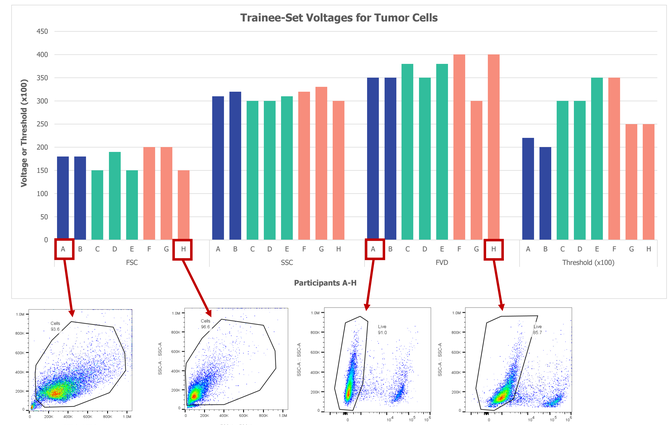




<u>CD45 neg Viability</u>
Viability gate: 89%
Whole Cells: 70%
Impedance: 80%



<u>CD45 pos Viability</u>
Viability gate: 94%
Whole Cells: 91%
Impedance: 87%



All participants were given the same direction by the same instructor; to adjust detector voltages so that the majority of events were within the range of the FSC and SSC parameters, repeat the same so that all events are in the range of the fluorescent parameter, and adjust the signal threshold so that <10% of total events were debris while ensuring that some of the debris was still recorded and distinguishable from cell events. Shown is the variation in instrument settings among participants.

Looking Forward

The imaging capability of the CytPix has proven its immense value for use as a teaching tool in addition to enabling quick, visual sample quality assessment. For future directions we would like to have an evaluation of basic theoretical concepts before and after the workshop to assess any improvement in core concept demonstrated. Furthermore, with such a large number of samples being generated that are from the same source there are many possibilities to test user-dependent and protocol-dependent variation like sample storage stability and common marker staining intensity, frequency, and stability. We are also seeking suggestions on simple ways to utilize this technology for future troubleshooting, education, and workshops.