CENTRIFUGE-LESS IMMUNOSTAINING OF SUSPENSION CELLS FOR FLOW CYTOMETRY ANALYSIS USING THE DROPARRAY™ SYSTEM

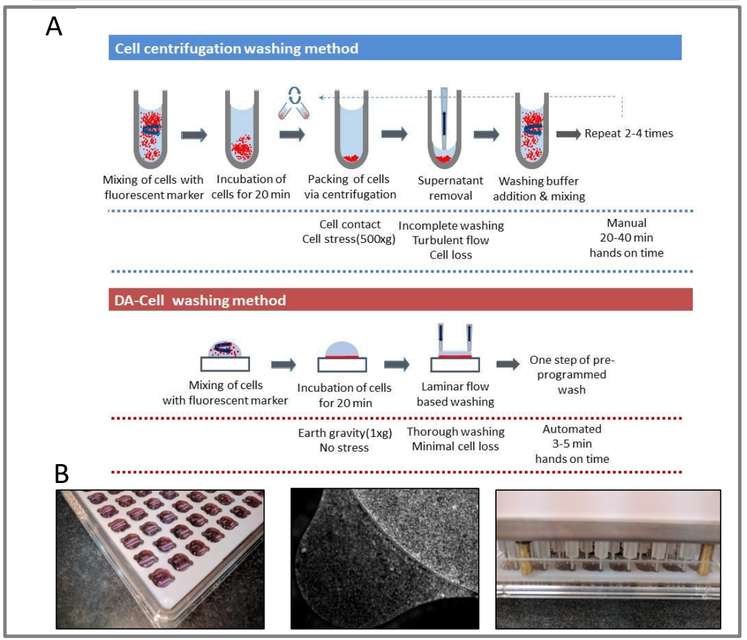
Kong Leong Cheng, Ih Chin Kon, Ralph Lim, Melvin Lye, Xavier Le Guezennec and Namyong Kim from Curiox Biosystems, San Carlos, CA 94070

*Weili Xu, Joni Chong, Brian Abel and Anis Larbi from A*STAR Singapore Immunology Network, Singapore*

PURPOSE:

The preparation of samples for flow cytometry typically involves staining of cells with fluorescently-conjugated antibodies that enable the detection of cell populations of interest and their corresponding phenotypes. The standard preparation for flow cytometry analysis requires a centrifugation step to spin down the cells and enable the removal of unbound antibodies. However, such a washing procedure is inherently stressful on the cells and may have an impact on cell integrity and viability. Here, we present a new convenient methodology to wash suspension cells based on unique laminar flow properties of DropArray plate technologies, DA-Cell. This technology offers greater than 95% retention of suspension cells without a centrifugation step. As an automated method, it performs washing (2-4 minutes) and minimises hands-on time to 3-5 minutes compared with 20-40 minutes for a centrifugation based method. Additionally, DA-Cell automation generates consistent and reproducible data independent of an operator. Here, we compared DA-Cell and a conventional centrifugation method in the processing of peripheral blood mononuclear cell (PBMC) samples for flow cytometric analysis.

Figure 1: (A) Comparison of centrifuge vs DA-Cell wash method, (B) Droplet on DA-Cell plate, (C) Close-up of a DA-Cell µwell and (D) Close-up of DA-Cell washer.



METHOD:

PBMC samples were subjected to a conventional staining preparation with Annexin V/ Propidium Iodide or a custom 21-antibody panel in FACS tube or U-bottom microtiter plate. In parallel, the same sample was prepared with DA-Cell using a 96-drop based plate format and equivalent ratios of antibodies and cell numbers as the classical preparation. For removal of unbound antibody, classical sample preparation used a 4X centrifugation based wash while DA-Cell sample preparation used 1 X DA-Cell wash cycle of 4 minutes. Samples were analyzed on BD FACSymphony™. Multi-color and multi-parametric analysis was conducted using both Cytofkit Bioconductor package and Flowjo.

Figure 2: Assessment of PBMC viability after washing with DA-Cell or Microtiter plate (MTP) methods: Analysis of (A) DA-Cell or (B) MTP methods, and (C) Summary statistics of 5 samples.

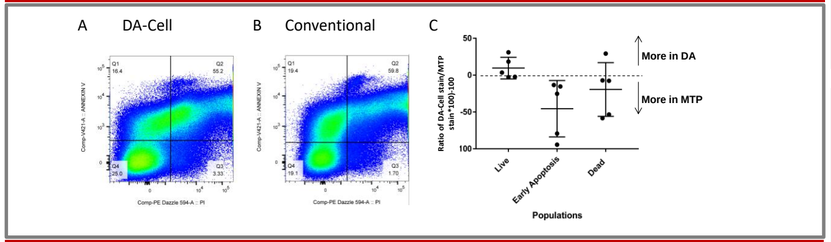


Figure 3: A comparison of flow cytometry cell population separation & frequencies after staining PBMCs with a multi-color panel using a 2ml FACS tube based or DA-Cell based wash.

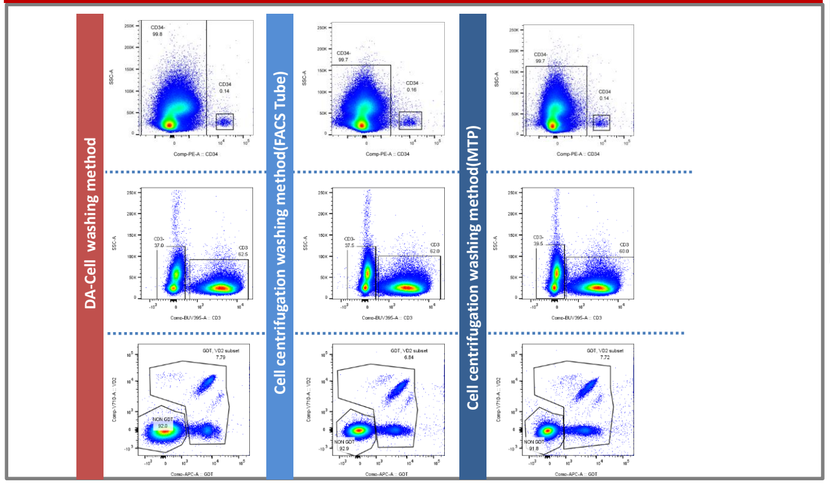
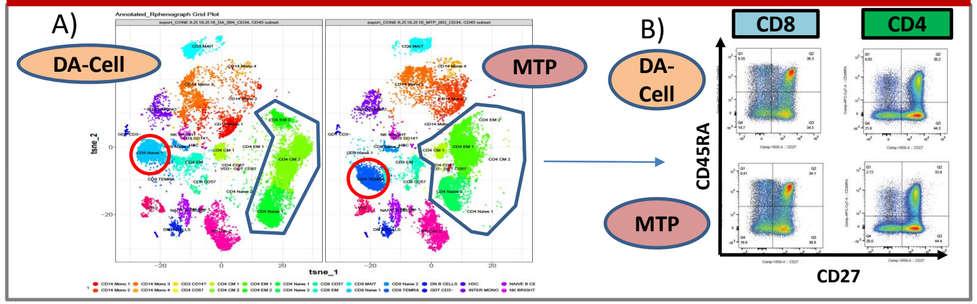


Figure 4: A) Unsupervised multi-parametric data analysis of a 21-antibody panel using combined t-SNE (Cytofkit) on PBMCs processed with either DA-Cell based wash or MTP. B) Actual FACS Plot of the Sample



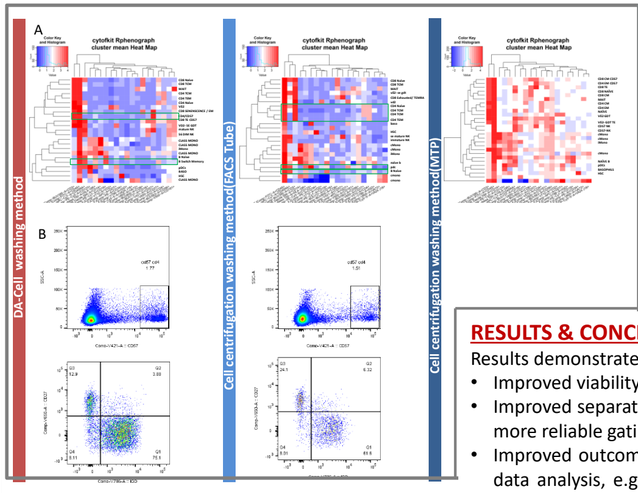


Figure 5: (A) Heat map of unsupervised clustering based on single t-SNE plots analysis. (A) DA-Cell based heatmap defines a cluster for CD4+ CD57+ and switched memory B cells absent in a centrifuge based washing.

RESULTS & CONCLUSION:

Results demonstrate that DA-Cell technology delivers:

- Improved viability of cells (Fig 2),
- Improved separation between cell populations leading to more reliable gating of distinct cell subsets (Fig 3).
- Improved outcomes with unsupervised multi-parametric data analysis, e.g. detection of CD4+CD57+ or switched memory B cell subsets in DA-Cell data but not in classical wash procedure data (Fig 4&5).
- Overall DA-Cell benefits include: improved cell viability, retention >95% cells regardless of the number of washes, easier segregation of cell populations, consistent & reproducible data even without an operator, 3-5 minute washing, and improved output of high-dimensional data.

With minimal hands on time, DA-Cell technology enables researchers to conduct complex multi-parametric flow cytometry cell assays without the cellular stress and time associated with centrifugation based procedures.