Cell identity, count, and viability for critical quality attributes using the Cellaca PLX image cytometer

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Cell and Gene therapy is one of the fastest growing fields for cancer therapeutics that heavily relies on robust and consistent instrumentations and technologies to verify and validate the critical quality attributes (CQA) that are fit-for-purpose. Some of the key parameters required for satisfying CMC (Chemistry Manufacturing and Controls) criteria standards for cellular therapeutic products are cell identity, count, viability, purity, potency, stability, and microbiological quality, which may be routinely performed using flow cytometry. Some of these critical attributes can be exceedingly trivial for flow cytometric analysis, which can be complex, requires a dedicated user, and may carry a high maintenance cost. In the past decade, affordable image cytometry has been developed for cell characterization and cell-based assays but has not demonstrated the sensitivity required for visualization and analysis of surface markers, cell health, and viability. In this work, we demonstrate the capability of the newly developed Cellaca PLX image cytometry system for population analysis of surface markers, viability of fluorescent protein expressing cells, and cell health in comparison to flow cytometry. For immunophenotyping assays, PBMCs were stained with anti-human CD3/CD4/CD8 antibodies with Hoechst. Additionally, viability detection of GFP and RFP-containing cell lines was performed by staining with Far-Red Dead and Hoechst. Finally, apoptosis was induced in Jurkat cells with staurosporine and stained with Caspase 3-488, Far-Red Dead, and Hoechst. All assays were imaged and analyzed using the Cellaca PLX image cytometer, and the data generated were

compared directly to the CytoFlex flow cytometer. Results show similar percentages of surface marker populations: CD3 (76% and 81%, PLX and CytoFlex, respectively), CD4 (43% and 42%), and CD8 (15% and 17%). RFP and GFP-containing cells showed comparable viabilities on Cellaca PLX and CytoFlex, andthe population percentages of Hoechst positive cells stained with Caspase 3 and Far-Red Dead were within 5% of each other. Our experiments demonstrate that the Cellaca PLX can be of significant value to the Cell and Gene Therapy communities providing novel image cytometry methods which may satisfy several CMC criteria including high-throughput, high sensitivity, and low maintenance.