Integration of High-Resolution Imaging Capabilities with Traditional Flow Cytometry

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INTRODUCTION

Coupling imaging with flow cytometry offers new opportunities for sample quality evaluation and cell characterization. The aim of this work was to integrate high speed, high resolution brightfield imaging to existing flow cytometry workflows in our research laboratories.

These assays evaluated cellular changes following chemical stimulation, fluorescent quantitation of chimeric antigen receptor T (CAR-T) cell targeting of B cell lymphoma cells, side-by-side comparisons of potential sample preparation protocols and cell line contamination evaluation. In each of these diverse procedures, we found high speed imaging capabilities beneficial in improving downstream analysis and data quality with no requirements for special sample preparation and minimal impact on data collection time.

MATERIALS AND METHODS

All data acquired on Invitrogen™ Attune™ CytPix™ Flow Cytometer following compensation with single stained controls where appropriate. Unless otherwise noted, all data collected with BRV6Y default configurations. No changes to existing sample preparation steps were utilized for imaging work – samples were prepared and recorded at concentrations already in use for these assays.

Adjustments to gating strategies have become part of our routine workflow on the Attune CytPix cytometer. Data presented from aggregate events found in CAR-T cells (provided by Thermo Fisher Scientific) recorded at 1000 µL/minute. Upstream processing of cells for immunophenotyping panel not detailed in this work.

The early effects of chemical stimulation on T-lymphocytes was evaluated by analyzing E6-1 Jurkat cells (ATCC) 4 hours after the addition of 50 ng/mL Phorbol 12-myristate 13-acetate (PMA, Sigma) and 1 µg/mL ionomycin (Thermo Fisher Scientific) to growth media (10% HI FBS RPMI). Data was collected at 200 µL/minute after resuspension in PBS.

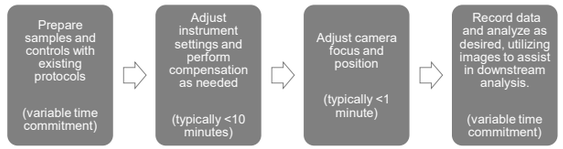
Bacterial contamination of cell cultures is presented here in Ramos cell cultures (originally sourced from ATCC, grown in house in 10% HI FBS RPMI). This contamination was discovered during unrelated immunophenotyping work with this cell line (panel not shown). Data collected at 100-200 µL/minute.

Biochemical and morphological changes during camptothecin-induced cell death was also evaluated in E6-1 Jurkat cells (ATCC). To do so, cells were incubated with 10 µM camptothecin (Sigma) at 37ºC in growth media (10% HI FBS RPMI), washed twice in PBS and stained with propidium iodide (Thermo Fisher Scientific) and Alexa Fluor™ 488 conjugated Annexin V in Annexin V binding buffer, per manufacturer instructions (Thermo Fisher Scientific). Data was collected at 100 µL/minute.

CAR-T cell targeting of CD19 tumor cells was visualized following independent staining of Ramos cells (ATCC) with 5 mM Invitrogen™ CellTrace™ Violet and CAR-T cells (provided by Thermo Fisher Scientific) with 1 mM Invitrogen™ CellTrace™ Far Red and co-incubated for 1 hour at 37ºC. Data was collected at 200 µL/minute.

Following several intracellular staining, fixation and storage options, morphology of E6-1 Jurkat cells (ATCC) was characterized at 100 µL/minute to compare sample preparation options both for target staining indices and for preservation of cell quantity and quality.

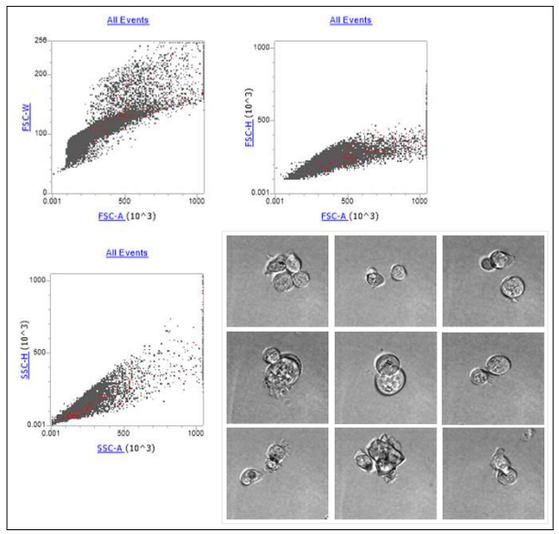
Figure 1. Sample preparation and data collection workflow



The workflows used to collect this data on the Attune CytPix were not remarkably different than those used on conventional Attune cytometers. The additional preparation step (camera focus and position adjustments) was performed in the same software interface, allowing us to rapidly progress to data recording once samples were prepared.

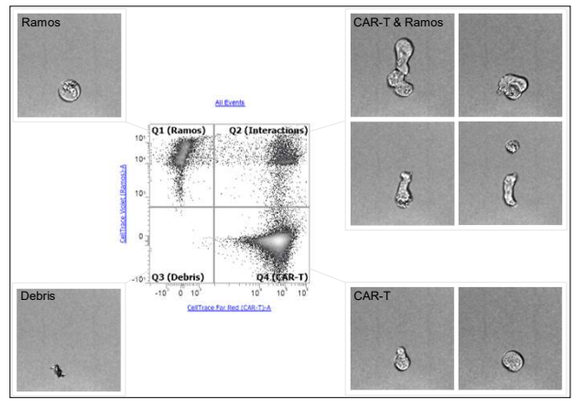
RESULTS

Figure 2. Images provided means to quickly improve gating choices without sacrificing panel options or run settings



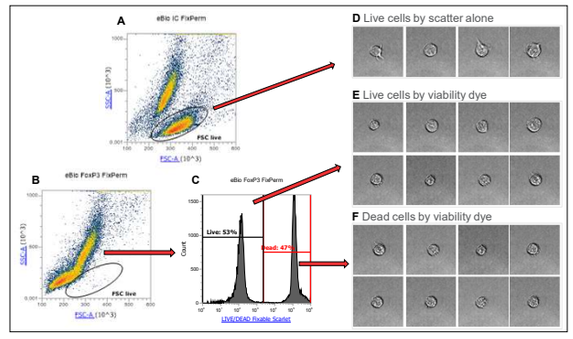
Approaches to debris and aggregate removal have been adapted for several assays after utilizing image backgating to identify problem areas. Above, CAR-T cells run at 1000 µL/minute were briefly evaluated in the CytPix image view gallery and several aggregate events (~50, red dots) backgated onto three doublet exclusion plot options to guide the best approach for this data set. Representative examples of the backgated images are shown.

Figure 3. Imaging-assisted cytometry data supports fluorescence-based identification of B-cell lymphoma targeting by CAR-T cells



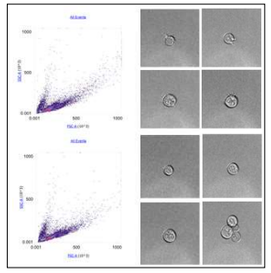
Brightfield images clearly detail populations identified by intracellular labels without additional sample prep. After staining of individual populations with CellTrace™ Violet or CellTrace Far Red and co-incubation for 1 hour at 37ºC, data in Q1 shows Ramos cells without CAR-T cell interactions, Q2 shows cell-cell interactions between these two populations, Q3 contains debris and finally Q4 shows CAR-T cells.

Figure 4. Brightfield imaging confirms cell viability determination



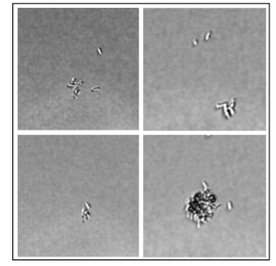
Some methods enable live and dead cells to be roughly distinguished by scatter alone (A), while other treatments do not (B). Addition of a LIVE/DEAD Fixable Viability dye improves live/dead discrimination (C). Importantly, this assessment of cell viability can be confirmed by their differing morphology using brightfield imaging provided by the Attune CytPix (D-F).

Figure 5. PMA and ionomycin reduce surface protrusions of Jurkat cells



Images demonstrate that modest changes in scatter profiles are likely caused by or indicative of reduction in surface protrusions. Attune CytPix images demonstrate morphological changes that were not immediately obvious from flow cytometry data alone.

Figure 6. Rapid detection of sample contamination during routine analysis



Imaging speed and event rate allowed us to identify low levels of contamination during routine analysis. This proved vital in tracing potentially contaminated subcultures. Images of early (left) and later (right) contamination.

CONCLUSIONS

Including high speed image collection enhanced our team's efforts to investigate the biological relevance of existing flow cytometry protocols. Without the requirement for additional sample preparation steps or separate equipment, we are now able to capture high resolution images along with our multicolor flow cytometry data. Here we have showcased a selection of these efforts, including the utilization of images to further define data analysis gates, confirmation of fluorescent data in immune synapses, characterization of populations which are depleted in candidate protocols, the effects of procedural treatments on cell morphology with minimal sample preparation and large event rates, and finally the value of imaging data in quality control measures of cell cultures and other biological materials.

These results demonstrate the integration of brightfield imaging capabilities into a wide range of existing protocols without loss of data breadth or quality.

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