BECKMAN COULTER
Life Sciences

Fully-Automated Cellular Analysis by Flow Cytometry

Summary

- Direct integration of CytoFLEX Flow Cytometer to Biomek i-Series Automated Workstations enables complete automation of sample processing and data acquisition.
  - CytoFLEX, a small footprint bench top analyzer, can collect 15 parameters with high sensitivity.
- Automated the plating, drug treatment, trypsinization, and staining of cells for apoptosis and cytotoxicity analysis
  - Selective tip pipetting enabled serial dilutions and processing of partial plates for time course studies
- Measured dose and time responses for multiple compounds in both suspension and adherent cell lines

Flow cytometry is a widely-used and powerful tool for single-cell analysis – an essential ability for those studying heterogeneous cell populations. However, the need for cells to be in single-cell suspensions can result in challenging sample preparation. This can include trypsinization of adherent cells and/or centrifugation steps to remove staining reagents. Automating these steps can decrease the time at the bench while improving reproducibility by ensuring consistent treatment (i.e. trypsin incubations) across samples. In addition, moving to a plate-based format increases the potential sample throughput.

Here we demonstrate how the Biomek i7 Automated Workstation (Figure 1A) was used to automate the complete cellular workflow for induction and analysis of apoptosis in two cancer lines. The Biomek instrument utilized its HEPA-filtered enclosure to maintain cell sterility during manipulations. In addition, the i-Series instruments enable simple and direct integrations, including the CytoFLEX Flow Cytometer configured with a plate loader (Figure 1B) used here, without the need for additional robotic transports.

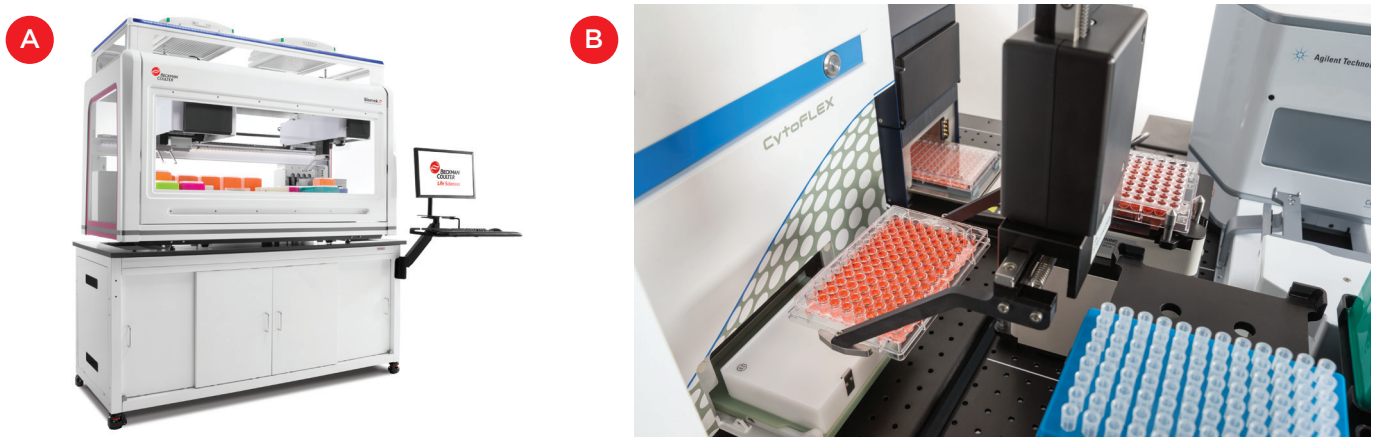

Figure 1. Biomek i7 Automated Workstation with HEPA filters (A) accessing an integrated CytoFLEX Flow Cytometer with plate loader (B).

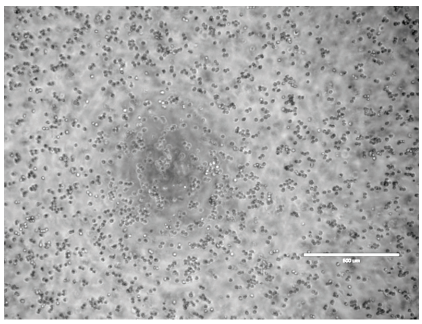

Figure 2. HCT116 cells following automated trypiniszation and resuspension.

We chose human leukemia (Jurkat) and colon carcinoma (HCT116) cell lines to demonstrate the workflows for both suspension and adherent cells. In both cases 25,000 cells were plated in 96-well plates and after 24 hours, the selective tip feature of the multichannel head was used to serially dilute three compounds – staurosporine, camptothecin, and 5-fluorouracil. These apoptosis inducers were added to cells and incubated for 24-72 hours. Prior to staining, the HCT 116 cells were trypsinized, using an on-deck Peltier heating device for incubation and the multichannel head was used for repeated pipetting to create a single-cell suspension (Figure 2). Both cell lines were incubated with CellEvent® Caspase-3/7 Green (Life Technologies) to identify cells undergoing apoptosis and DRAQ7 (Beckman Coulter) to label cells with compromised cellular membranes as a measure of cell death.

Cells were identified using forward and side scatter and apoptosis and cell death stains were measured in the FITC and PC5.5 fluorescence channels respectively. Analysis plots were generated in Kaluza 1.5 software. Figure 3A shows the viable, apoptotic, and dead HCT116 cells for a high, medium, and low dose of 5-fluorouracil treatment at 48 hours, and Figure 3B shows the 48 hour dose response curve and calculated IC50s for all three compounds, illustrating the effectiveness of the automated serial dilutions. Figure 4A shows the progression of Jurkat cells through the cell death pathway over time following a treatment with 78 nM camptothecin. The change in the percentage of cells in each condition is plotted in Figure 4B. This drug time course was made simple by multichannel selective tip pipetting, which enabled the cells to be plated once, the drug dilutions stamped into replicate wells, and one set of wells be harvested per time point.

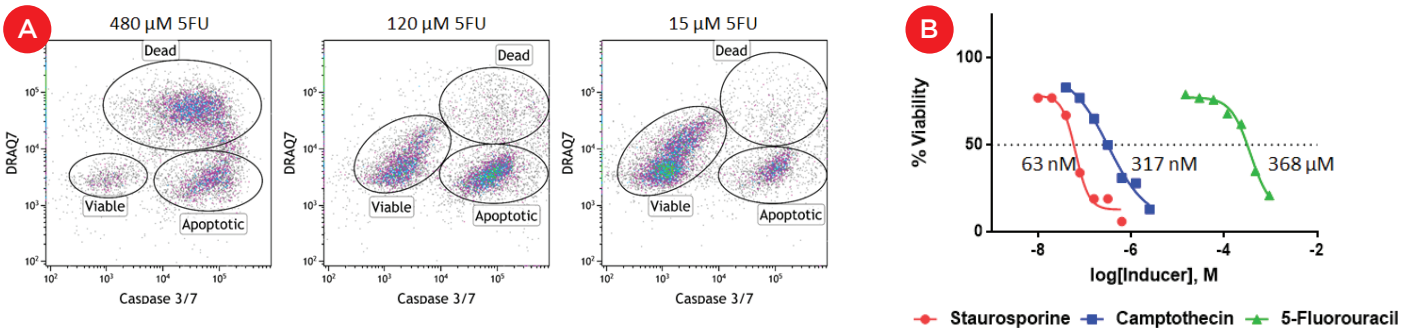

Figure 3. A) Dot plots showing HCT116 cell populations that are viable (unstained), apoptotic (caspase 3/7 positive), or dead (DRAQ7 positive) following 48 hours of 5-fluorouracil treatment. The cytotoxic effects are diminished as concentration decreases indicating effective serial dilutions. B) Dose response curves and IC50 values based on the percentage of viable HCT116 cells following 48 hour treatment with three apoptosis inducers.

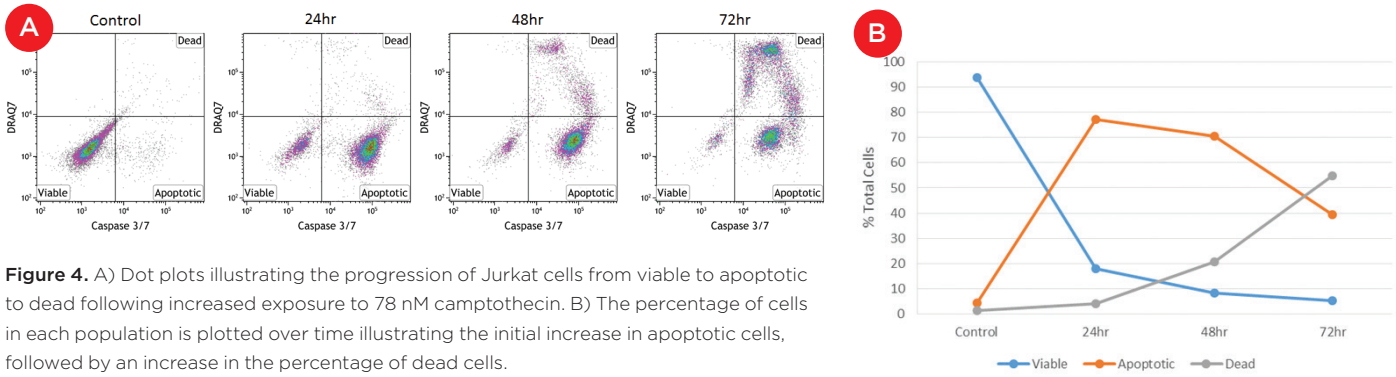

Figure 4. A) Dot plots illustrating the progression of Jurkat cells from viable to apoptotic to dead following increased exposure to 78 nM camptothecin. B) The percentage of cells in each population is plotted over time illustrating the initial increase in apoptotic cells, followed by an increase in the percentage of dead cells.

While the reagents used here did not require washes, the ability to directly integrate microplate centrifuges (Figure 1B), plate washers, and incubators to the i-Series instruments means that antibody-based workflows that discriminate populations in a heterogeneous mixture can also be easily automated. In addition, if samples need to be processed by a tube-based flow cytometer, the Span-8 pipettors can be used to rapidly process samples in tubes or perform a final transfer from plates to tube prior to analysis. Finally, for high throughput applications SAMI EX software can be used to schedule staining and analysis workflows to ensure consistent treatment across plates.

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*Data obtained during development.

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