Fully-Automated Cellular Analysis by Flow Cytometry

Shilpa Parakh, Michael Kowalski, Jason Lowery, Nancy Dempsey, Tara Jones-Roe
Beckman Coulter Life Sciences, Indianapolis, IN

OVERVIEW

- Direct integration of a CytoFLEX Flow Cytometer to the Biomek i-Series 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

INTRODUCTION

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 Workstation 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.

AUTOMATED CELLULAR ANALYSIS

Figure 1. Automated cellular analysis. The Biomek i7 Automated Workstation with HEPA filters (A) was directly integrated with a CytoFLEX Flow Cytometer (B), enabling the Bimoek grippers to access the plate loader feature of the CytoFLEX (C).

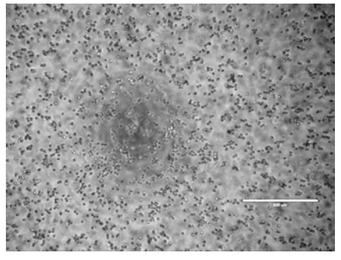

Figure 2. Automated single cell suspension. Image of adherent HCT116 cells following automated trypsinization and resuspension. The multichannel head was used to repeatedly pipette wells to ensure single cell suspensions.

RESULTS

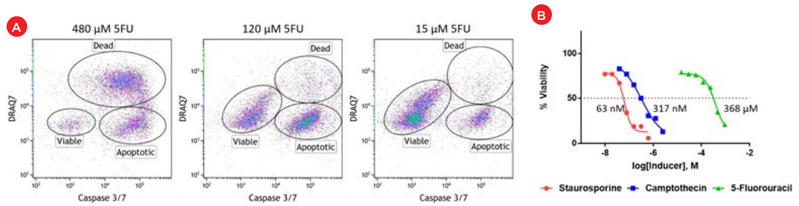

Figure 3. A) Dot plots showing HCT116 cell populations that are viable (unstained), apoptotic (caspase 3/7 positive), or dead (DRAQ7 positive) following 48 hours of 5-fluorouracil treatment. The cytotoxic effects are diminished as concentration decreases indicating effective serial dilutions. B) Dose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

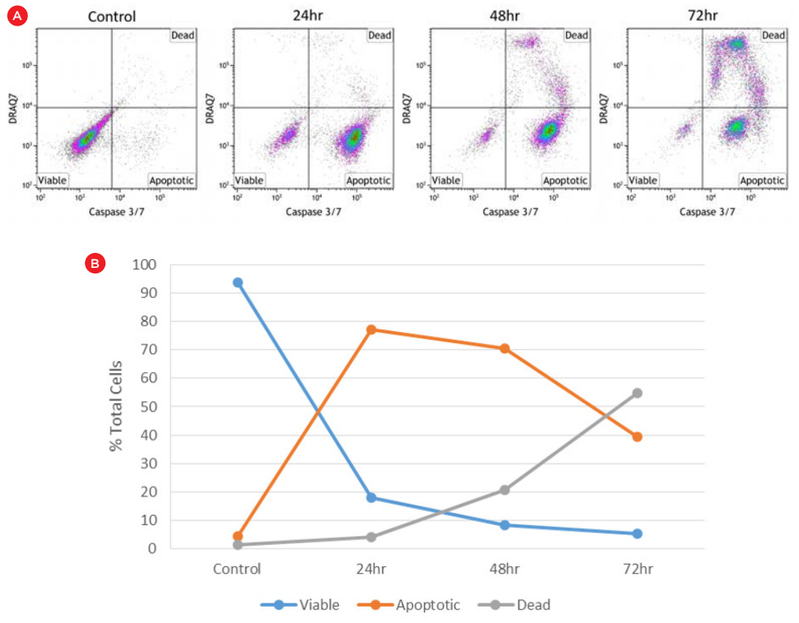

Figure 4. A) Dot plots illustrating the progression of Jurkat cells from viable to apoptotic to dead following longer exposure to 78 nM camptothecin. This time course was made simple by multichannel selective tip pipetting, which enabled the cells to be plated once, the drug dilutions stamped into replicate wells, and one set of wells be harvested per time point. 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.

MATERIALS AND METHODS

CELLS AND REAGENTS

We used human leukemia (Jurkat) and colon carcinoma (HCT116) cell lines to demonstrate the automated workflows for both suspension and adherent cells. Cells were cultured in RPMI 1640 and McCoys 5a media respectively with 10% FBS.

Three compounds - staurosporine, camptothecin, and 5-fluorouracil – were used to induce apoptosis. Cells were incubated with serial dilutions or a DMSO control for 24-72 hours prior to analysis.

CellEvent® Caspase-3/7 Green (Life Technologies) was used to identify cells undergoing apoptosis and DRAQ7 (Beckman Coulter) was used to label cells with compromised cellular membranes as a measure of cell death.

AUTOMATED CELL TREATMENTS

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 the three compounds.

Following incubation and prior to staining, HCT116 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). 10 µL of combined stains were then added to all wells using a single column of tips on the multichannel head.

FLOW CYTOMETRY ANALYSIS

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.

CONCLUSION

- To demonstrate a fully-automated workflow for cellular analysis by flow cytometry, we have shown:
  - The Biomek i7 Automated Workstation reliably automated the creation of drug dilutions and treatment and staining of cells for apoptosis analysis
  - Drug dose response and time course studies illustrate the abilities of selective tip pipetting for partial plate processing.
  - Direct integration of the CytoFLEX Flow Cytometer with plate loader functionality
- Possible workflow variations can include:
  - The ability to directly integrate microplate centrifuges (Figure 1C), plate washers, and incubators in addition to the CytoFLEX Flow Cytometer means that antibody-based workflows that discriminate populations in a heterogeneous mixture can also be easily automated on the Biomek i-Series instruments.
  - The Biomek Span-8 pipettors can be used to rapidly process samples in tubes or perform a final transfer from plates to tubes prior to analysis on a tube-based cytometer.
  - For high throughput applications SAMI EX software can be used to schedule staining and analysis workflows to ensure consistent treatment across plates.

*Data obtained during development
For Research Use Only. Not for use in diagnostic procedures

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at beckman.com
AAG-2355PST02.17