Multiparametric analysis of cell health status using flow cytometry

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ABSTRACT
Cellular responses to drug treatment can be varied and heterogeneous. Often, a multiparametric approach is necessary to identify cellular pathways that are responding or affected. To maximize the number of parameters analyzed per sample, we aimed to combine several functional sensors into one assay. In addition, we set out to streamline the workflow focusing on live cell responses. To this end, we designed a flow cytometry-based detection system centered on a single incubation condition for sensors that have minimal spectral overlap. Analyzing Jurkat cells treated with cancer chemotherapeutic agents that trigger cellular apoptosis, we were able to simultaneously detect the loss of cells in G2/M phase using the stoichiometric DNA dye, Hoechst 33342, an increase in apoptotic cells with a sensor for activated caspase 3/7 (CellEvent Caspase-3/7 Green); the loss of mitochondrial membrane potential with tetramethylrhodamine, methyl ester (TMRM); and an increase in dead cells as well as cells in late apoptotic stage, using a SYTOX viability dye. We also tested another four sensor combination that focused on detecting cellular stress. Upon prolonged camptothecin induction, we were able to detect an increase in mitochondrial stress with MitoSOX, a sensor that detects mitochondrial oxidative stress; an increase in cellular stress with a CellROX reactive specific for reactive oxygen species. There was also a loss in mitochondrial membrane potential as well as a greater loss of cellular viability. In summary, using a model of cellular response to drug treatment, we performed multiparametric cell status analysis with a combination of cellular detection reagents on a flow cytometer.

INTRODUCTION
Determination of cell status has often been limited to single parameter analysis. This approach limits the number of parameters examined, usually resulting in sequential testing using different reagents that target distinct biological readouts. Compounding the limited throughput of a single readout assay, single parameter analysis has the potential to miss out on other confounding biological effects. Flow cytometry lends itself to multiparametric analysis. In this study, we highlight the use of flow cytometric analysis in detecting four different cell status parameters in a single analytical run. One set of sensor will detect cell cycle state, induction of apoptosis, mitochondrial health and cell viability. The second set will determine the stress state of the cell by measuring mitochondrial stress, cellular stress, mitochondrial health and cellular viability. These sensors are marketed as single parameter reagents, but in this study, we show that four different sensors are compatible in live cell staining to demonstrate cellular health. Furthermore, we are able to identify hits from a compound library using a combination of these sensors on a flow cytometer.

MATERIALS AND METHODS
Verification testing: Jurkat cells were resuspended at 1 x 10⁶ cells/ml and 1 ml of cells were stained with one of the cell cycle state, induction of apoptosis, mitochondrial health and cell viability sensors and one of the stress sensors. Cells were incubated with these for 30 minutes at 37°C with 5% CO₂. After this period, cellular responses were analyzed using an Attune NxT Acoustic Cytometer.

Treatmet for compound library analysis: For this test, 10,000 cells in 80 ul of RPMI 1640 + 10% fetal bovine serum were plated into each well of a 96 well plate. Compounds from one plate of the Toosli screen Mini (Toosli, Cat No.2890) were used. The initial 10 ml stock was diluted to 100 uM. This allowed for a 1:10 addition of the compounds to 90 uM of cell to result in the final compound concentration of 10 uM. Incubation of the compounds with the cells were carried out in 37°C with 5% CO₂ for 18 hours. After which, components of MultiKit 1 and MultiKit 2 were added to the final assay concentrations. Cells were returned to a cell culture incubator for 30 mins prior to flow cytometric analysis, using an Attune NxT Acoustic Cytometer with the attached AttoSammemeter.

RESULTS

Figure 1. Individual cell status sensors can be used in combination

Figure 2. Individual cell status sensors to detect cellular stress

Figure 3. Screening with Toosli Screen Mini compound library

Figure 4. Responses from compound library analysis

CONCLUSIONS
- Single parameter detection reagents can be used in combination to create multparameter kits.
- Spectrally distinct reagents allows for experimental setup without compromising fluorescence compensation.
- Multiparameter kits can be tailored to determine cellular responses in drug compound library analyses.

REFERENCES

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