Use of Multi-Parametric Analysis and Image Drill Down to Facilitate the Study of Cell Cycle and Apoptosis Modulators

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Abstract
High content analysis is a powerful methodology for assessing cell cycle and apoptotic status at the cell by cell level. It is easy to multiplex with specific markers, and provides valuable information with that is not accessible using analyses at the well or plate level. Taking full advantage of the possibilities of high content analysis is important when planning experiments and data analysis. In this study we demonstrate how Molecular Devices Total Imaging Solution can be used to rapidly characterize cell cycle modulators. The Library of Pharmacologically Active Compounds (LOPAC) was applied to DU145 cells for 48 hours. Staining with specific reagents to permit the evaluation of cell cycle and apoptotic status was followed by image analysis with AcuityXpress™ and ImageAnalysis™. The application module identifies and classifies all DNA-containing structures (apoptosis, late mitosis, Early mitosis, G2/M and S phase) while the classification criterion for mitosis was set by specifying the intensity above local background of the DNA stain (Hoechst) or the mitotic-specific stain (anti-phospho H3ser10). After a 48 h incubation at 37°C (5% CO₂), the cells were fixed, permeabilized and stained with antibodies against phospho Histone H3 (blue), PARP in green and phospho Histone H1S10 in red. (BOTTOM) The application module identifies and classifies all DNA-containing structures (apoptosis, late mitosis, Early mitosis, G2/M and S phase).

Molecular Devices’ Total Imaging Solution

Figure 1: Assay Development

Assay Development (Experiment Figures 1 and 2a)

Figure 1a: Configuration of the Cell Cycle Application Module

The Cell Cycle application module provides accurate image segmentation of nuclei and cells. The Cell Cycle application module is easy to use: settings can be interactively adjusted via the histogram and scatter plot.

1. The Cell Cycle application module provides accurate image segmentation of nuclei and cells.
2. The Cell Cycle application module is easy to use: settings can be interactively adjusted via the histogram and scatter plot. Images taken at 10x or 20x magnification give comparable results (data not shown but available on the web-site link above).
3. Identification of mitotic cells was comparable using either DNA average intensity or mitotic-specific probes as a marker (data not shown but available on the web-site link above).

Figure 2: Conventional Analysis of Dose Response and LOPAC Screen using AcuityXpress

The LOPAC analysis takes advantage of the rich dimensionality of the HCS dataset. (a) Cell-Dividing Max (SDM) was calculated for the objects in the experiment illustrated in panels (A) and (B) (bottom). In addition to providing good separation of profiles, (b) Principal Component Analysis (PCA) identifies up to 6 profiles (components) contributing to the most variance in the data. All the compounds are plotted relative to the top 3 components. Grouped compounds together are likely to have similar profiles. The control compounds staurosporine and Taxol, are indicated in brown and red, respectively.

Figure 3: Multi-Parametric Analysis of LOPAC Screen using AcuityXpress

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Material and Methods
Assay Development (Experiment Figures 1 and 2a)

Figure 1b: Imaging and Segmentation using the Cell Cycle Application Module

CDFS Overlay of the Image from the 3 different channels (nucleus in blue, PARP in green and phospho Histone H1S10 in red). (BOTTOM) The application module identifies and classifies all DNA-containing structures (apoptosis, late mitosis, Early mitosis, G2/M and S phase).

5. After a 48h incubation at 37°C (5% CO₂), the cells were fixed, permeabilized and stained with antibodies against phospho Histone H3, cleaved PARP and cell nuclei were detected as described above.

Image Analysis (AcuityXpress® with MetaXpress Acquisition Module)

Figure 2a: Determination of Dose of Compounds in the LOPAC Screen

Additional information is available in previous posters upon request or on the web (http://www.moleculardevices.com/product_literature/family_links.php?productID=113).

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Figure 3a: Determination of Dose in the LOPAC Screen

Results of multiple types of analysis can easily be compared. Here, compound in the PCA have been colored with the results of the analysis with (a) 2-D plots or (b) SOM Clusters. Good separation of colors in figure 5b suggests that multi-parametric analysis separates complex phenotypes better than the conventional analysis.

Figure 5: Comparing Multiple Analysis Methods - PCA

Figure 6: MDCEarth Enables Bi-Directional Interaction Between Image Data and Analysis Results

The MDCEarth analysis of AcuityXpress provides high quality images for analysis with the Cell Cycle application module, which allows the extraction of multi-parametric data. Direct and real-time interaction between the data and the images is possible with the MDCEarth feature in the AcuityXpress software.

Conclusions
The Total Imaging Solution from Molecular Devices offers all the tools for the HCS analysis of Cell Cycle and Apoptosis. Imagepress™ provides high quality images for analysis with the Cell Cycle application module, which allows the extraction of multi-parametric data. Direct and real-time interaction between the data and the images is possible with the MDCEarth feature in the AcuityXpress software.