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

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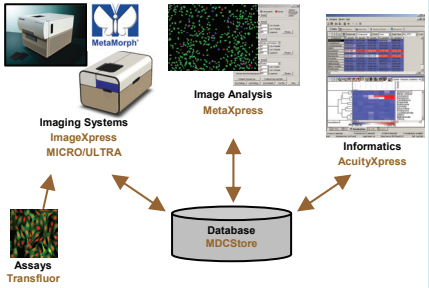
Abstract

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 cancer research investigators with information that is not accessible using analysis at the well or at the population level. Taking full advantage of the possibilities of high content analysis requires a set of integrated tools for image acquisition, image analysis 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 Pharmaceutically 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 acquisition with ImageXpress^{MICRO} and image analysis with the MetaXpress Cell Cycle application module. The AcuityXpress 2.0 cellular informatics software has been designed to enable multi-parametric analysis tasks as well as full interaction between the image data and the numerical data. We demonstrate how these features are powerful tools to rapidly separate complex phenotypes, characterize cell cycle/apoptosis modulators and interact with the image data for quality control before and after analysis.

Molecular Devices' Total Imaging Solution



Material and Methods

Assay Development (Experiment on Figures 1 and 2a)

1. DU145 cells were plated in each well of a 96-well plate at a density of 8,000 cells/well and incubated for 24 h at 37C (5% CO_2) .
2. A range of concentrations of Taxol (0.005-0.5 µM) or Staurosporine (0.02-2 µM) was applied to the cells.
3. After a 24 h incubation at 37C (5% CO_2), the cells were fixed, permeabilized, and incubated with antibodies against phospho Histone H3ser10 (Upstate) and cleaved PARP (Cell Signaling Technologies).
4. The anti phospho Histone H3ser10, anti-cleaved PARP and cell nuclei were detected with an Alexa Fluor 555 goat anti-rabbit secondary antibody (Invitrogen), an Alexa Fluor 488 goat anti-mouse secondary antibody (Invitrogen) and Hoechst 33342 (Invitrogen), respectively.

LOPAC Screen (Experiment on Figures 2b, 3, 4, 5)

1. DU145 cells were plated in each well of 16 96-well plates at a density of 8,000 cells/well using an AquaMax DW4 (Molecular Devices).
2. The FLIPR dispensing head was used to dispense compounds from a Library of Pharmacologically Active Compounds (LOPAC, Sigma; 1 µM final), Taxol or Staurosporine (at 3 concentration each) in the wells.
3. After a 48 h incubation at 37C (5% CO_2), the cells were fixed, permeabilized and incubated with antibodies against phospho Histone H3ser10 (Upstate) and cleaved PARP (Cell Signaling).
4. The anti phospho Histone H3ser10, anti-cleaved PARP and cell nuclei were detected as described above.

Image Acquisition (ImageXpress^{MICRO} with MetaXpress Acquisition)

1. Plates were loaded using a CRS robot (Thermo/Fisher)
2. 4 sites were imaged in each well using a 10X Plan Fluor objective (Nikon) and DAPI, FITC, and Texas Red filter cubes (Semrock).

Image Analysis with Cell Cycle Application module (MetaXpress)

1. The Hoechst image was used as the DNA content source map.
2. The range of sizes of the nuclei as well as their intensity above local background were specified.
3. 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 Histone H3ser10).
4. For the apoptotic stain (anti-cleaved PARP), the stained area, range of sizes of nuclei and their intensity above local background were specified.
5. The classification results were previewed and thresholds were interactively adjusted.
6. Image per image as well as cell by cell measurements were selected (13 measurements per images and 8 measurement for each individual cell can be selected in the Cell Cycle module).
7. The analysis was applied to all the images.

Data Analysis using AcuityXpress 2.0

Conventional and multi-parametric analysis as well as interaction with the image data were performed with AcuityXpress 2.0

Figure 1: Assay Development

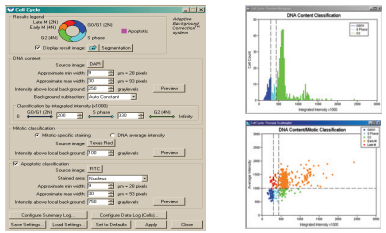


Figure 1a: Configuration of the Cell Cycle Application Module
A specific mitotic marker or the DNA average intensity can be used to determine mitotic cells. In addition, a specific apoptotic marker can be used to determine apoptotic cells. The DNA Content Classification Histogram and the DNA Content/Mitotic Classification Scatter plot are tools that can be used to interactively adjust the settings.

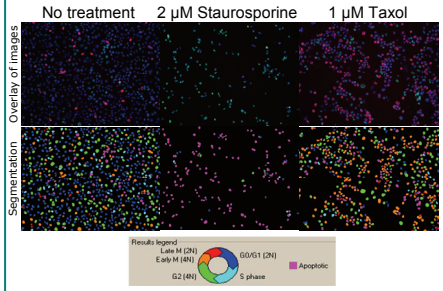


Figure 1b: Imaging and Segmentation using the Cell Cycle Application Module
(TOP) Overlay of the images from the 3 different channels (nuclei in blue, PARP in green and phospho Histone H3S10 in red). (BOTTOM) The application module identifies and classifies all DNA-containing structures (Apoptotic, Late Mitosis, Early Mitosis, G2, G0/G1 and S phase).

Assay development conclusions

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

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 histograms and scatter plots.

3. Images taken at 10x or 20x magnification give comparable results (data not shown but available on the web-see link above).

4. 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-see link above).

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

In conventional data analysis, a limited number of parameters (typically one or two) are taken into consideration. AcuityXpress features a complete toolset for conventional analysis. (a) Curve fitting of dose responses allows analysis of IC_{50} and EC_{50} and reduction of the data to these values. (b) Compounds (X axis) are plotted against the % late mitotic cells (Y). An overlay with the Apoptotic Hits (in orange) shows compounds with an effect on both mitosis and apoptosis (Taxol, vincristine and vinblastine are the 3 orange compounds on the right). Both curve fitting and plotting methods are reduction methods (i.e. they reduce the number of dimensions of the analysis).

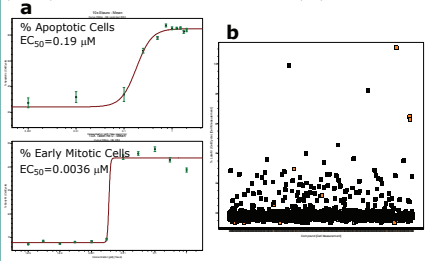


Figure 3: Multi-Parametric Analysis of LOPAC Screen using AcuityXpress

Multi-parametric analysis takes into account all the dimensions of the HCS dataset. (a) Self-Organizing Maps (SOMs) allow clustering of the objects (in this case, compounds) based on the similarity of their multi-parametric profile. (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. Compounds grouped together are likely to have similar profiles. The control compounds staurosporine and Taxol, are indicated in brown and red, respectively.

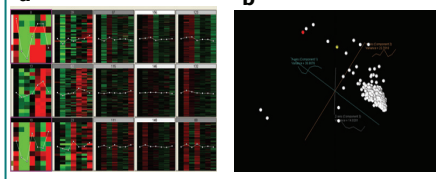


Figure 4: Comparing Conventional and Multi-Parametric Analysis- SOM

Results of conventional (i.e. one or two parameters) and multi-parametric analysis can easily be compared. Here, compound in the SOM clusters (RIGHT) have been colored with the results of the analysis with 2-D plots (LEFT)

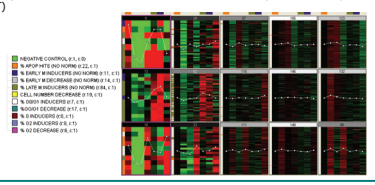


Figure 5: Comparing Multiple Analysis Methods- PCA

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.

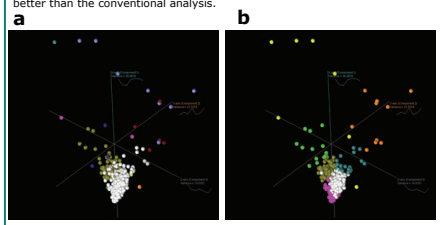
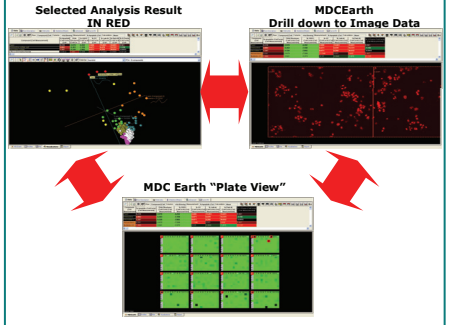


Figure 6: MDCEarth Enables Bi-directional Interaction Between Image Data and Analysis Results

The MDCEarth™ feature of AcuityXpress allows direct interaction of image and numerical data at all levels and in both directions. This is a very powerful tool for quality control of the data analysis or to determine if further analysis is required. (Top left) Selection of objects (here Taxol, vincristine and vinblastine) in the data analysis (here the PCA view) allows immediate visualization of the corresponding image data at any level in MDCEarth such as the heat map/plate level (bottom), the high resolution image level (top right) or the individual cell level (not shown). Identification of new phenotypes can also be easily performed using reverse selection from MDCEarth to the data analysis.



Conclusions

The Total Imaging Solution from Molecular Devices offers all the tools for the HCS analysis of Cell Cycle and Apoptosis. ImageXpress^{MICRO} provides high quality images for analysis with the Cell Cycle application module, which allows the extraction of multi-parametric data. Data analysis in AcuityXpress is complete with conventional analysis tools as well as multi-parametric methods. Direct and real-time interaction between the data and the images is possible with the MDCEarth feature in the AcuityXpress software.

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