High Content Analysis of a Robust 384-Well Cell Migration Assay

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Introduction

Quantifying cell migration in an objective manner is of interest to researchers studying cancer metastasis and wound healing. The Oris™ Pro 384 Cell Migration Assay (Platypus Technologies) is a first-in-class, automatable, 384-well high throughput screening assay. By using a novel approach to image analysis, combining the ImageXpress® Velos laser scanning cytometer (Molecular Devices) and FCS Express 4 Image Cytometry™ (De Novo Software), we were able to rapidly analyze the effects of cell seeding density and inhibitors on the motility of three different cell lines.

Methods

Oris Pro 384 Cell Migration Assay

The Oris Pro 384 Cell Migration Assay utilizes a centrally located, self-dissolving, biocompatible gel (BCG) to form a uniformly sized, cell-free detection zone on cell culture surfaces. HT-1080, MDA-MB-231 and HUVEC cells were seeded into 384-well plates in 20 μL volumes and patterned in an annular monolayer surrounding the BCG (Fig. 1a) (Table 1). After a 90 min incubation to allow the BCG to dissolve and the cells to attach and spread (Fig 1b.), 20 μL aliquots of medium containing Cytochalasin D (0.0078 μM – 2 μM, final concentrations) or 0.1% DMSO were added to the wells and the cells allowed to migrate for 16 hours (Fig. 1c) into the newly revealed detection zone. Post migration, cells were fixed with 0.25% glutaraldehyde and labeled with TRITC-phalloidin for detection with the ImageXpress Velos (Fig. 2a) Dose response experiments using the actin polymerization inhibitor Cytochalasin D were conducted on the cell lines.

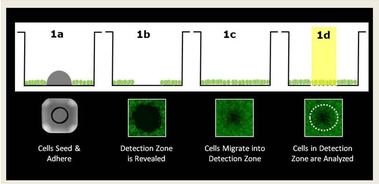

Table 1

Cell Line	Seeding Density (Cells/Well)
HT-1080	5,000
HT-1080	9,000
HUVEC	4,500
MDA-MB-231	5,000

Figure 1-Schematic of Oris Pro Cell Migration Assay. Cells are seeded and adhere (1a), the BCG dissolves revealing detection zone and cells are treated (1b), cells migrate into detection zone (1c), cells in detection zone are analyzed (1d).

Imaging of Cell Migration by the ImageXpress Velos Cytometer

The ImageXpress Velos platform (Fig. 2a) allows fast and simple cellular imaging and analysis of assays in a multi-well plate format. Whole well images are captured using either single or dual laser excitation with up to 4 detection channels (including scatter) per laser. Entire plates (6 to 1536-well) can be scanned in < 5 minutes while simultaneous image segmentation and measurement provides multiplexed cell-by-cell analysis. The segmented images and associated data generated by the ImageXpress Velos were imported into FCS Express 4 Image Cytometry.

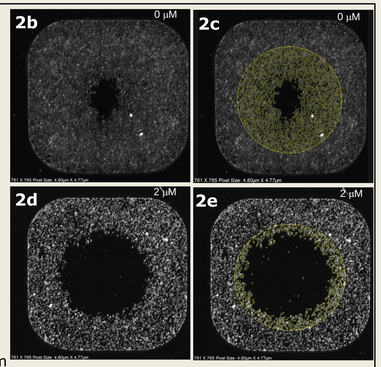

Figure 2-The ImageXpress Velos platform and image acquisition. Following fixation and staining, the Velos (Fig. 2a) provided high speed acquisition of images and assay data (Fig. 2b-2e). Raw images were acquired at 5x5 μm resolution using a 488 nm laser and 510-540 nm emission filter. Image XpressVelos software enabled on the fly image segmentation and measurement of size, shape, and intensity for the cells that migrated into the detection zone (yellow overlay, Fig. 2c and 2e). Fig. 2b and 2d show representative 0.1% DMSO negative control and 2 uM cytochalasin D positive control raw images, respectively while Fig. 2c and 2e display the corresponding segmented images.

Parameter Math Formula Strategy

Equation 1- $D = \sqrt{(Cx - Rx)^2 + (Cy - Ry)^2}$

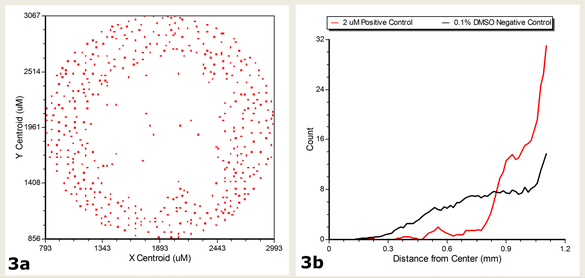

Figure 3-Parameter Math Formula. In Eqn 1. D represents the distance, in μM, of each cell from the center of the detection zone. Cx,y represent the X,Y coordinate of the center of the detection zone (in μM). Rx,y represent the X,Y coordinate of the centroid of each object region (in μM). Fig. 3a shows the Rx, Ry plotted for each cell. Fig. 3b shows a histogram overlay of the positive and negative controls plotted as Distance from Center (converted to mm in FCS Express 4 Image Cytometry) of the detection zone. (Plots represent: HT1080-9k/well, fig. 3a. - 0.125 μm, fig. 3b.-controls).

Data Analysis with FCS Express 4 Image Cytometry

Axes in FCS Express 4 Image Cytometry were calibrated to actual distances based on ImageXpress Velos calibrations. Distances from the center of the detection zone were calculated with parameter math based on the equation in fig. 3. Four zones of migration were defined by applying interactive gates to measure the degree of migration for each treatment based on picture plots of negative (0.1% DMSO) and positive (2 μM Cytochalasin D) controls (fig. 4). Statistics were generated using a combination of customizable and predefined formulas via tokens and were batch exported to Microsoft Excel for further analysis and archiving.

Image and Plot Gating Strategy

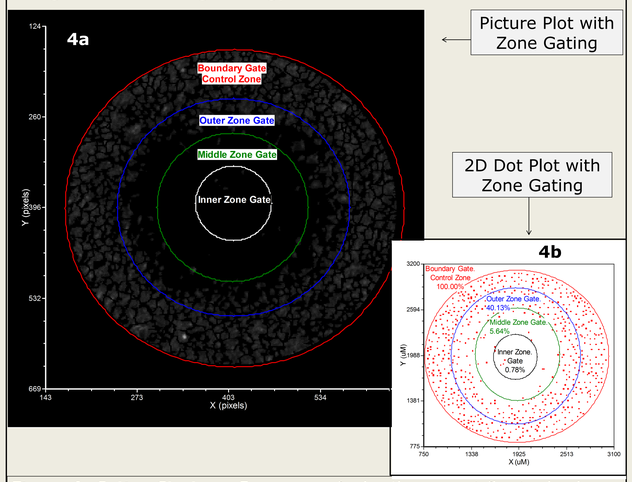

Figure 4- Gating Strategy. Four zones of migration were defined using image and 2D plot gating in FCS Express 4 Image Cytometry. The boundary gate (control zone) represents the edge of the 2 mm detection zone created by the BCG while the outer, middle, and inner zone gates were defined arbitrarily by the user based on the assay and kept constant for a particular plate. Fig. 4a and fig. 4b show the gates on a representative picture plot and 2D dot plot respectively.

Results and Discussion

Speed and reproducibility:

The Oris Pro 384 Cell Migration Assay allowed continuous visual assessment of cell motility throughout the duration of the experiment. The Oris Pro Assay enabled reproducible preparation of 192 wells corresponding to a 10 point titration series with 4 replicates of 4 cell lines/seeding density in a reproducible manner. The ImageXpress Velos provided high speed, whole well image acquisition, segmentation, and analysis. Scanning of 192 wells in 384 well format was achieved in approximately 4 minutes.

High Content Plate Based Analysis:

The combination of the Oris Pro Assay and acquisition via the ImageXpress Velos allowed for high content analysis of assay results in FCS Express 4 Image Cytometry with heat maps. Fig. 5 shows heat maps of the assay in which a dose response for Cytochalasin D can be clearly delineated via number of cells. A heat map displaying distance from center of the well assists in visualizing data and finding replicate wells that fall outside the normal range. As expected, a higher dose of inhibitor results in a greater average cell distance from the center of the detection zone. Representative picture plots and histograms of positive and negative controls assisted in quickly determining the success of the assay for 2 of 3 gating strategies as represented by Z' scores calculated in FCS Express.

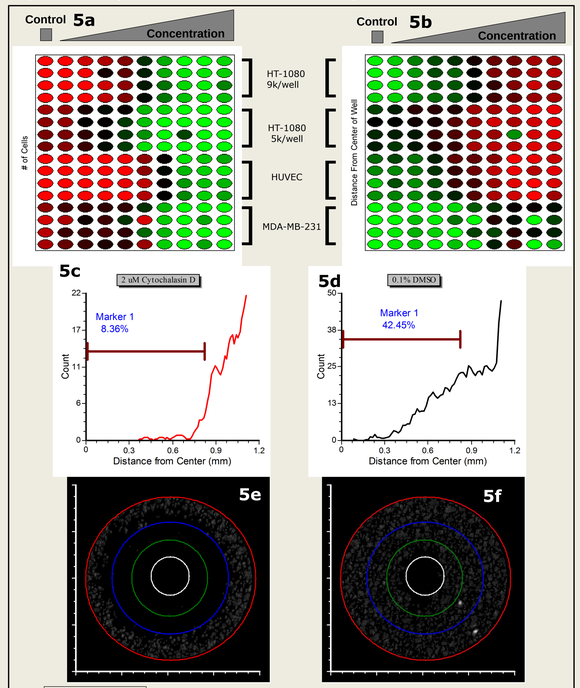

Table 2

Zone	2 μM # Events	0.1% DMSO # Events	Z' Score
Outer	24	434	0.84
Middle	5	138	0.52
Inner	1	11	-2.60

Figure 5-Heat Maps, Picture Plots, and 2D Dot Plots. Data was loaded into FCS Express 4 Image Cytometry and displayed in custom configured heat maps with red representing higher values and green representing lower values. The heat map displaying number of cells (Fig. 5a) confirmed the dose response while the custom parameter math formula (fig. 3) was applied to the second heat map (fig. 5b) to view data as distance from center of the well. Control samples were displayed in histograms (markers indicate percent of total migration as defined by the positive control Fig. 5c.) and picture plots (fig. 5c-5f) for confirmation of gating strategies. The number of events for each gate and associated Z' score were calculated to validate each gate in the assay (Table 2). The results indicate that the inner detection zone data should be disregarded, likely because of the small number of events recorded.

Results and Discussion

Batch Analysis in FCS Express 4 Image Cytometry:

Batch processing in FCS Express was used to quickly analyze all samples in the assay. Results for the mean distance from the center of the well and the percentage of cells in each zone gate were exported for each well (Fig. 6). Increasing the concentration of inhibitor generally results in a reduction in the percentage of cells in each zone. The batch actions and template for analysis were saved to easily reproduce the results and analysis in future experiments.

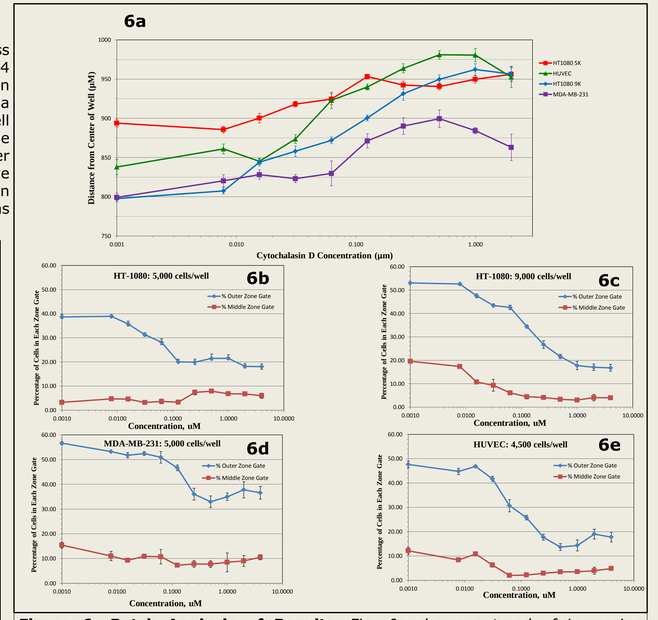

Figure 6- Batch Analysis of Results. Fig. 6a shows a trend of increasing distance from the center of the plot (inhibited migration) for all cell lines and conditions. The greatest effect was observed in HUVEC cells. In Fig 6b-e the percentage of cells in each zone gate was calculated and plotted against concentration (the inner zone was excluded based on a low Z' score (fig. 5). HT1080-9k/well and HUVEC cells exhibited the greatest decrease of cells in both outer and middle zone gates over the titration series indicating inhibition of migration. HT-1080-5k/well and MDA-MB-231 cells showed a less dramatic inhibition. Migration rate was determined to be a factor of both cell type and seeding density.

Conclusions

Oris Pro 384 Cell Migration Assays are easy to use, robust and fully automatable while providing real-time visibility of cells and cellular response to treatment with migration inhibitors.

The ImageXpress Velos provided whole well imaging of the migration assay plate with a resolution that allowed clear segmentation of individual cells. Image acquisition and on the fly analysis were performed in ~ 4 minutes.

FCS Express 4 Image Cytometry allowed objective definition of positive and negative control gates based on image and multi-parametric data.

Parameter Math and a variety of plotting options allows FCS Express users to calibrate plotted data to actual distances while visualizing and gating on raw images, histograms, or 2D plots.

A combination of the Oris Pro 384 Cell Migration Assay, ImageXpress Velos, and FCS Express 4 Image Cytometry is an attractive option for high throughput screening of modulators of cell motility.

Acknowledgements

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