

Oris™ Pro Cell Migration and Invasion Assays run on IN Cell Analyzer 2000

HaiGuang Zhang¹, Keren I. Hulkower² and Robert Graves¹

¹GE Healthcare, 800 Centennial Ave, Piscataway, NJ 08855; ²Platypus Technologies, LLC, 5520 Nobel Dr., Suite 100, Madison, WI 53711, e-mail: Robert.Graves@ge.com

Introduction

Cell migration and invasion both play important roles in many physiological and pathological processes such as wound healing and metastasis of cancer cells¹. As these processes provide potential molecular targets for the development of novel therapeutics, there is significant effort to develop physiologically relevant assays suitable for high-throughput screening of compound libraries². High-content imagers like GE Healthcare's IN Cell Analyzer 2000 are ideal instrumentation platforms for these applications by providing high-throughput multi-wavelength image acquisition at single-cell resolution, coupled with sophisticated image analysis software, thus enabling collection of multiple end-point data in a single study³.

The Oris™ Pro Cell Migration and Invasion Assays (Platypus Technologies, www.platypustech.com) are 96-well cell exclusion assays that may be useful for compound library screening. The assay plates are provided with spots of Biocompatible Gel (BCG) centrally deposited in each well to exclude cells from adhering in the centers of the wells. After the cells are seeded and allowed to adhere, the BCG self-dissolves to reveal reproducible cell-free Detection Zones in the center of each well, into which cells are then permitted to migrate (Figure 1). Cell movement can be monitored in 2-Dimensions across the surface of the plate (as in this study), or in 3-Dimensions when a Collagen I overlay is applied to permit invasion in the z-axis.

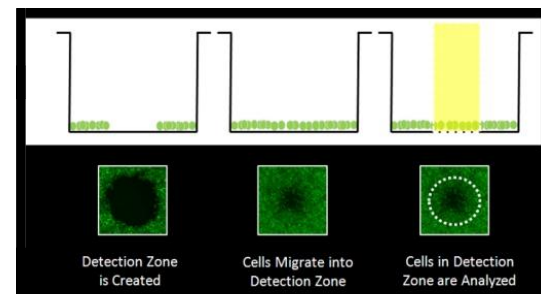


Fig. 1: Schematic of Oris™ Pro Cell Migration Assay

The ability to configure the IN Cell Analyzer 2000 with a camera containing a 2048 x 2048 pixel CCD makes this instrument very suitable for the imaging of 2D migration assays. This large camera coupled with use of a 2x or 4x objective, enables image capture at single cell resolution with a field of view (FOV) of 7.6mm² (2x) or 3.8mm² (4x). As shown in Figure 2, this allows whole-well image capture from a 96-well plate with the 2x objective, or capture of >70% of the well with the 4x objective. The FOV afforded by the large camera can result in a smaller number of image fields acquired per plate, thus increasing throughput.

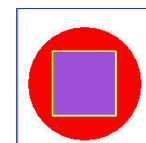


Fig. 2: Imaging area in a single well of a 96-well plate with IN Cell Analyzer 2000 (large CCD). The FOV with a 2x objective (blue outer box) is compared with FOV with a 4x objective (yellow box).

Methods

Human umbilical vein endothelial cells (HUVEC) were seeded at 25,000 cells/well onto Collagen I coated Oris™ Pro plates. After 1 hr, media was removed and replaced with fresh medium containing the actin inhibitor, Cytochalasin D (0.0078μM – 2μM), or 0.1% DMSO as a vehicle control. Cells were incubated for 18 hr followed by fixation (0.25% glutaraldehyde, 15 min), permeabilization (0.1% Triton-X 100) and staining with TRITC-phalloidin and DAPI. Images were acquired with IN Cell Analyzer 2000 using a 4x objective (1 image field per well).

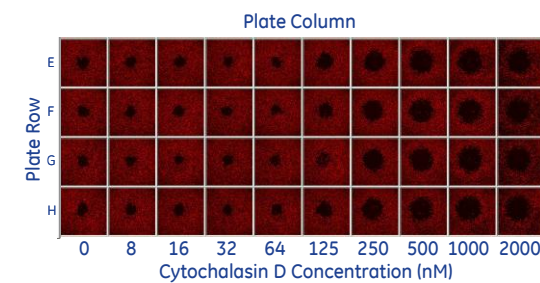


Figure 3: Image thumbnails with 4x objective of the TRITC-phalloidin-labeled cells. Rows E-F from the 96-well plate displayed.

Various image analysis strategies were explored for this assay using IN Cell Investigator analysis software (Level 3, flexible analysis). Firstly, the total area of each image was examined to provide whole image cell count and cell coverage. Secondly, a pre-migration reference image of the Detection Zone from the 2μM Cytochalasin D-treated wells was used to define a sampling region for measuring Detection Zone cell number and cell coverage in every image. By using appropriate object size filters in the analysis protocol, open area measurements were restricted to the large inner cell-free area, and any smaller open spaces between cells were not measured.

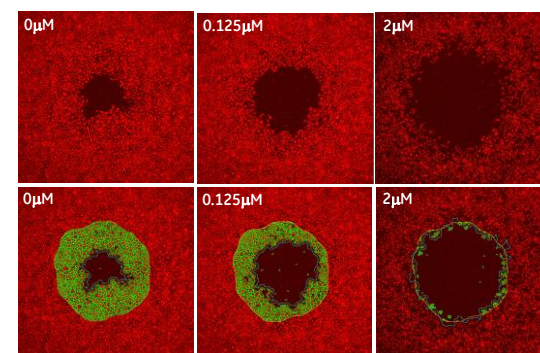


Figure 4: Example images of the TRITC-phalloidin-stained cells treated with Cytochalasin D. The lower row shows the image analysis overlays representing detection of the cell-free (open) area of the whole image (defined by the inner blue border). The outer border (yellow) is the edge of the sampling region defined from reference image of the Detection Zone. The green borders represent cell edges identified in the Detection Zone.

Results

The results compare image analysis strategies looking at the whole image, or by defining a sampling region based on the Detection Zone.

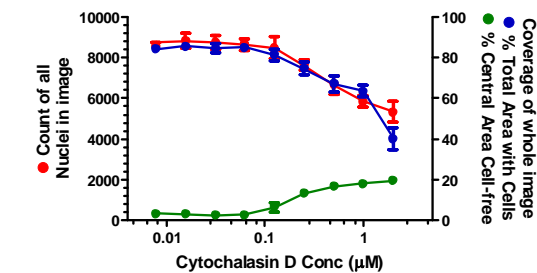


Figure 5: Results from analysis of the whole image. The cell count data is from the DAPI image channel and the coverage data is from the TRITC image channel. All data shown is +/- SD (n=8 replicate wells).

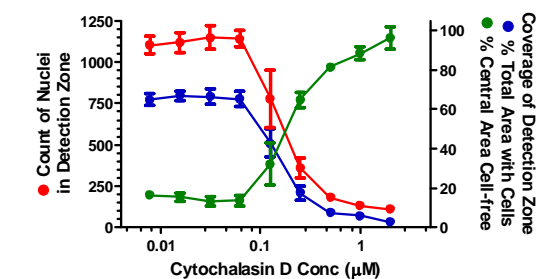


Figure 6: Results from analysis of the Detection Zone. The cell count data is from the DAPI image channel and the coverage data is from the TRITC image channel. All data shown is +/- SD (n=8 replicate wells).

Measurements from Whole Image		Z score
Count of Nuclei		0.34
% Area with Cells		0.54
% Area Cell-free		0.73
Measurements from Detection Zone		Z score
Count of Nuclei		0.79
% Area with Cells		0.82
% Area Cell-free		0.73

Table 1: Comparison of Z scores from analysis of the whole image or from analysis of the Detection Zone.

The results shown in Table 1 indicate that the assay delivers Z scores exceeding 0.5 for all analysis options, with the exception of the cell count from the whole image. By limiting analysis to the Detection Zone, the Z scores can be dramatically increased. One advantage of IN Cell Investigator software (Level 3 flexible analysis) is that all these measurements can easily be combined into one analysis protocol for comparative purposes.

Comparing data from sampling regions

By definition, High-Content Analysis allows capture of information-rich data from cellular assays. Staining nuclei with a DNA-intercalating stain like DAPI not only provides an image reference for determining cell number and position, but the nuclear DAPI signal can be used to monitor individual cell health and cell cycle status. In Figure 7 DNA content and nuclear texture was compared for wells treated with various concentrations of Cytochalasin D.

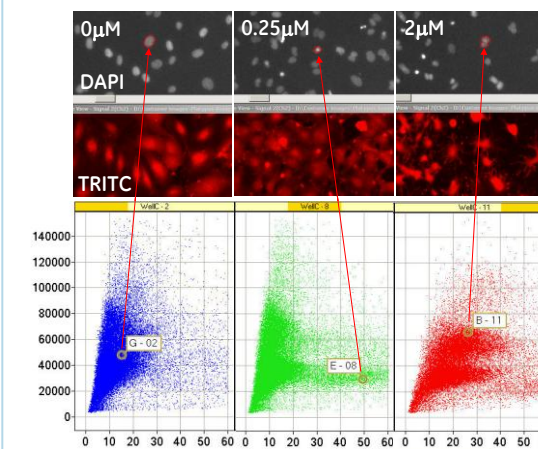


Figure 7: Comparison of nuclear DNA content and nuclear texture for wells treated with Cytochalasin D. Spotfire Trellis plots of intensity x area per nucleus (Y axis) and standard deviation of intensity (SD levels) per nucleus (X axis) from IN Cell Investigator analysis of cells treated with 0, 0.25 or 2μM Cytochalasin D. Each Spotfire plot is a composite from 8 replicate treatments, and each data point represents an individual cell. The Spotfire data display per cell can be directly linked to the IN Cell Investigator image display of that cell as shown.

The results show Cytochalasin D-induced effects on cell cycle progression thru an increase in cells showing the appearance of mitotic block. An additional advantage of using imaging for this assay is the ability to take measurements from specific populations of cells within the image. In this case the cells inside or outside the Detection Zone can be analyzed as independent populations for comparative purposes (Figure 8).

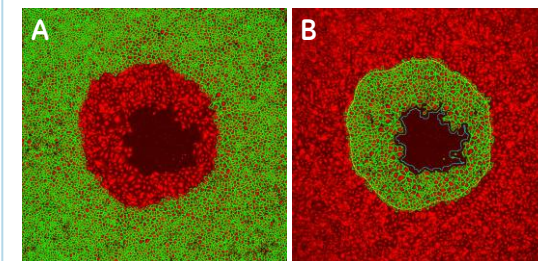


Figure 8: Analysis of cell sub-populations. The Detection Zone segmentation of the image (yellow boundary) allows cells (green) outside the zone (A) or inside (B) to be analyzed as separate populations. Both images show TRITC-phalloidin labeled cells.

Although not a definitive study, the data shows differences in nuclear texture and DNA content for cells inside and outside the Detection Zone, with the cells inside showing the characteristics of an actively dividing population (Figure 9).

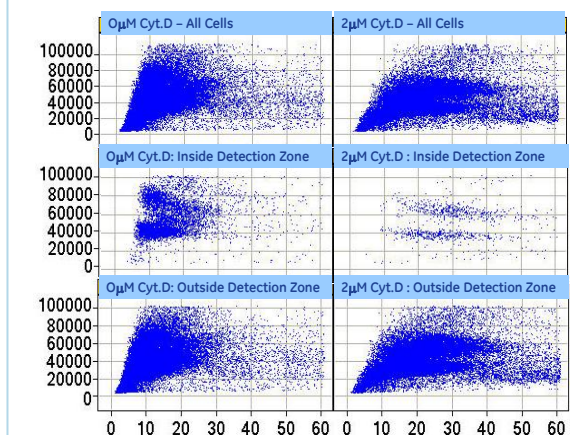


Figure 9: Comparison of nuclear DNA content and nuclear texture for wells treated with Cytochalasin D. Spotfire Trellis plots of intensity x area per nucleus (Y axis) and SD levels per nucleus (X axis) from IN Cell Investigator analysis of cells treated with 0 or 2μM Cytochalasin D. Each Spotfire plot is a composite from 8 replicate treatments, and each data point represents an individual cell. Data is displayed for all cells in the image (top) cells only in the Detection Zone (middle) and cells only outside the Detection Zone (bottom).

The results show effective use of IN Cell Analyzer 2000 High-Content imager with the 96-well Oris™ Pro Cell Migration Assay. Several formats of the assay have not covered here including analysis of cell invasion in 3D, monitoring cell migration kinetics in a time-course experiment and the newly introduced 384-well assay format. Although the 4x objective was used for image capture in this 96-well plate study, the 2x objective can be effectively used for whole-well imaging of this assay at single cell resolution (unpublished results). Higher power objectives can also be used but multiple image fields would have to be acquired to capture all of the Detection Zone, slowing imaging throughput.

Conclusions

- Use of the IN Cell Analyzer 2000 with large camera configuration for high-throughput study of cell migration using Oris™ Pro Cell Migration 96-well Assay plates, delivers results with high Z scores for a variety of assay metrics tested.
- Images acquired at 4x (or 2x) have sufficient contrast and resolution to allow analysis to single-cell level.
- The IN Cell Investigator analysis software allows user-defined study of cells throughout the whole image or can be performed on cells only in the Detection Zone for comparative study of the migrating population.

References

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- Carragher, 2009. Clin. Exp. Metastasis. 26:381-397.
- Thomas, 2010; J Biomol Screen. (1): 1-9.