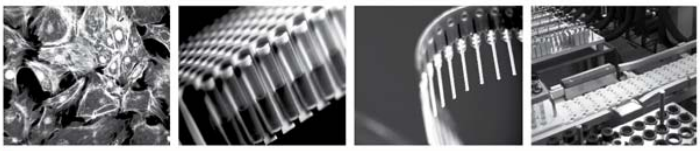

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Acumen eX3 Technical Note

A High Content, High Throughput 384-Well Assay for Cell Migration

Introduction

Cell migration plays a critical role in many physiological and pathological processes such as wound healing and cancer metastasis. It is therefore the goal of drug discovery programs to screen for therapeutic modulators that inhibit abnormal migration of cancer cells or promote migration of cells to stimulate wound healing.

Recent advances in laboratory automation and the development of high throughput, high content imaging systems have paved the way for contemporary large-scale drug screening paradigms to include cell migration assays (1). One of the most common cell migration assays utilizes the Boyden chamber which contains a porous polycarbonate or polypropylene membrane insert as a barrier through which cells migrate. However, the membrane insert obstructs the ability to visualize cell migration in real-time and thus limits use of this assay to capturing endpoint results (2), while the uneven distribution of the pores leads to variability in migration patterns and data analysis (3). Another popular cell migration assay, the scratch assay, utilizes a scraping tool to create a void in a confluent cell monolayer into which cells migrate. In this case, scratch assay results may be compromised by damage caused to the underlying extracellular matrix, release of factors from wounded cells and wound sizes that can be inconsistent making it difficult to ensure that the void areas are precisely created in all assay wells (3).

Platypus Technologies, LLC has developed the Oris™ Pro 384 Cell Migration Assay, a robust and completely automatable assay that utilizes a centrally located, self-dissolving biocompatible gel (BCG) to form a uniformly sized cell-free detection zone on tissue culture treated or collagen I coated cell culture surfaces. Cells are seeded into 384-well plates and pattern in an annular monolayer surrounding the BCG (Fig 1). Once the BCG dissolves, cells can migrate into the detection zone previously occupied by the BCG. Cells may be fixed and treated with multiple stains, including nuclear and cytoskeletal stains, to enable flexible data capture by enumerating migrating cells or calculating the area of closure within the detection zone.

Analyzing the Oris™ Pro 384 Cell Migration Assay with the Acumen eX3 instrument yields reliable and highly accurate cell migration data with high throughput capability. Key improvements of the Oris™ Pro 384 assays include: assay wells that are completely accessible to automated liquid handling equipment, unobstructed visualization of cell movement in real-time, and an HCI compatible platform that permits in-depth phenotypic data capture from assay wells using multiplexed cytostaining techniques.

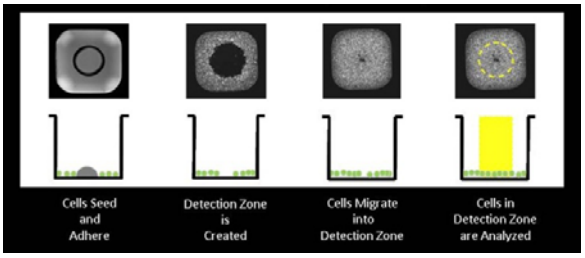


Fig 1. Schematic of Oris™ Pro 384 Cell Migration Assay.

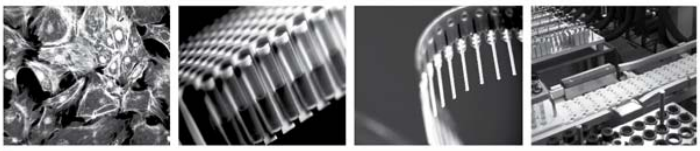
Acumen eX3

The Acumen eX3 is a laser-scanning, high throughput, high content imaging system that can scan with up to 3 lasers (405 nm, 488 nm and 633 nm) providing similar wavelength excitation to that of white light sources. PMTs detect up to 4 colours simultaneously. The application of image capture over a large area means that analysis is performed on an area, not a well basis. This equates to the simultaneous scanning of 4, 16 and 64 wells in 96, 384 and 1536 well formats, respectively.

Materials and Methods

Oris™ Pro 384 Cell Migration Assay

Human umbilical vein endothelial cells (HUVEC) were seeded at 4,500 cells/well in 20 µL of culture media in an Oris™ Pro 384 Cell Migration Assay – Tissue Culture Treated plate.



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After allowing 90 min at 37ºC/5%CO_2 for the cells to attach and spread, an additional 20 µL of media was added to wells containing the actin polymerization inhibitor Cytochalasin D or DMSO vehicle only. Cytochalasin D was tested at a range of concentrations between 0.0078 and 4 µM in 0.1% DMSO and the cells were allowed to migrate for 16 hr at 37ºC/5%CO_2. The cells were then fixed with 0.25% glutaraldehyde and stained with DAPI to visualize nuclei and TRITC-phalloidin in 0.1% Triton X-100 to label F-actin. The Oris™ Pro 384 Cell Migration Assay plate was scanned on an Acumen eX3 fluorescence microplate laser cytometer, with 1 µM Cytochalasin D treated wells serving as pre-migration reference wells, as described (4).

Scanning with the Acumen eX3

Plates were scanned at 0.5 x 8 µm resolution using 405 nm laser for DAPI staining (detecting with 420-470 nm filter set) and 488 nm laser for TRITC-phalloidin staining (detecting with 575 - 640 nm filter set). Migrated cells were defined using width and depth measurements of 2 – 100 µm and the "distance" object characteristic of 0 – 0.8 mm from the centre of the well.

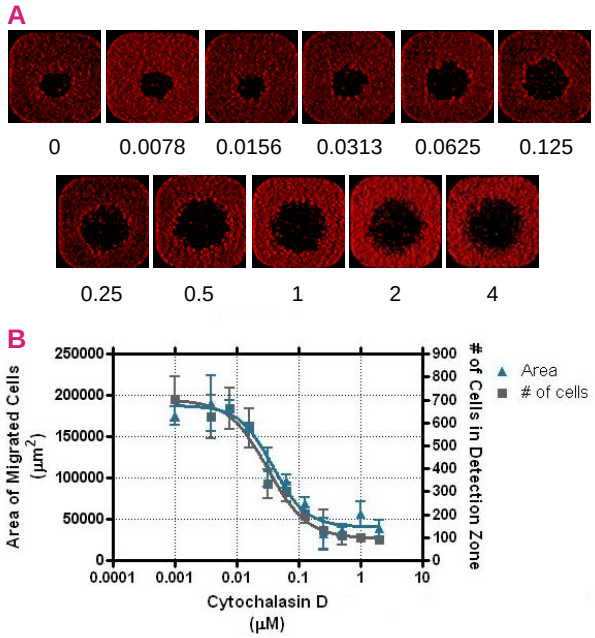

Fig 2. Effect of Cytochalasin D on HUVEC Migration. (**A**) Representative images of TRITC-phalloidin stained HUVEC cells in the presence and absence of Cytochalasin D (at indicated concentrations in µM) after 16 hr of migration as captured by an Acumen eX3. (**B**) Dose-response curves as calculated by area of migrated cells and enumeration of cells in the detection zone. The calculated IC_{50} values for area and cell number determinations were 0.038 and 0.032 µM, respectively. Data represents the average of 4 replicates per treatment condition ± SD.

Results and Conclusions

HUVEC migration was inhibited in a concentration dependent fashion in the presence of Cytochalasin D **(Fig 2)**. Migration was quantified using multiplexed cytostaining of cytoskeletal F-actin filaments and nuclei by either calculating the area covered by migrating cells stained with TRITC-phalloidin or by enumerating the number of DAPI-stained cells in the detection zone using the Acumen eX3. The two different methods for quantifying assay results yielded super-imposable dose response curves and substantially equivalent IC_{50} values of 0.038 µM for area determinations and 0.032 µM for cell number, respectively **(Fig 2B)**.

OrisTM Pro combined with the Acumen eX3 deliver High throughput migration assays

The Acumen eX3 is conducive for rapid data acquisition and simultaneous analysis of Oris™ Pro 384 Cell Migration Assays within 8 minutes per plate. The analysis template defining migrated cells can be setup within minutes to output desired parameters including number of migrated cells and total area of migrated cells. Acumen cytometric analysis software simultaneously scans the plate and analyses the data. When detailed image analysis is required, open source TIFF images can easily be generated whilst scanning.

References

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4. Hulkower, K.I. and Gehler, S. www.platypustech.com/ApNote_OrisPro_PreMigRef.pdf, Application Note accessed 2010-11-24.

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