

HTS performance and high-content analysis of antimigratory compound phenotypes using the Oris™ Pro 384 Cell Migration Assay

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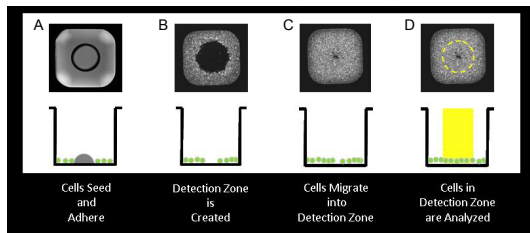
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Abstract

The discovery of antimetastatic agents that inhibit cancer cell motility has been hindered by a dearth of cell motility assays that are compatible with high-throughput screening (HTS). Here we report the HTS performance and utility of the Oris™ Pro Cell Migration Assay platform using the highly invasive MDA-MB-231 breast cancer cell line. This innovative cell migration assay utilizes a centrally located, self-dissolving, non-toxic biocompatible gel (BCG) to form a uniformly sized, cell-free detection zone. Using 384-well clear bottom microplates that are compatible with a variety of high-content screening (HCS) platforms, cells are seeded and allowed to attach in an annular monolayer surrounding the BCG. Once the BCG dissolves, cells can migrate into the detection zone previously occupied by the BCG. Using the number of cells that migrated into the exclusion zone as a primary readout, the assay was optimized and implemented on automated liquid handling equipment. The assay delivered Z-factors greater than 0.5 under both manual and automated processing conditions.

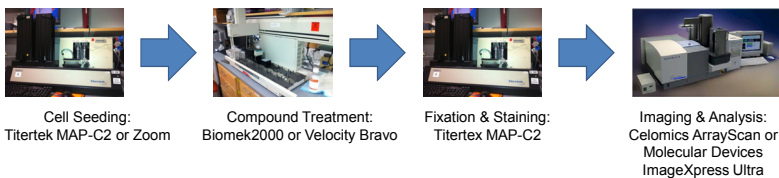
To exploit the assay's multiparametric capabilities, we quantified motility-associated activity profiles of six mechanistically distinct antimigratory agents. Upon compound treatment, cells exhibited dose-dependent loss of motility and assumed characteristic morphological phenotypes consistent with literature reports. Digital images were acquired at two different magnifications on a Thermo Scientific Cellomics® ArrayScan®, and analyzed with the Target Activation and Morphology Explorer Bioapplications. The assay delivered graded responses for all parameters tested (cell migration, chromatin condensation, cell density, nucleus area, cell size, actin content, and actin fibers) and accurately described the distinct cellular phenotypes instigated by the different antimigratory agents. The data demonstrate that the Oris™ Pro 384 Cell Migration Assay is compatible with automated liquid handling systems and HCS instrumentation and can satisfy HTS performance criteria.

Assay Schematic

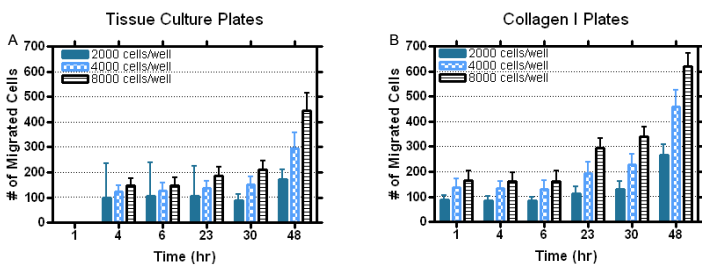


A) Cells are seeded and adhere in an annular monolayer surrounding the Biocompatible Gel (BCG). B) BCG dissolves to reveal a cell-free Detection Zone. C) Cells migrate into the Detection Zone. D) Image cells via microscopy or High Content Imagers. Images are then analyzed for cell migration as well as phenotypical changes.

HTS Workflow



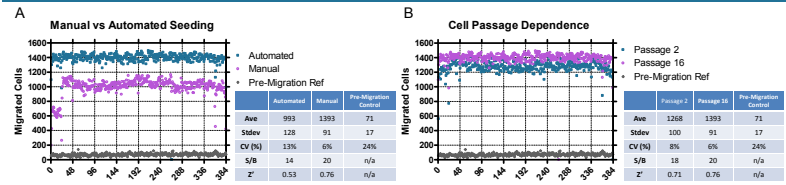
Assay Development: Seeding Density



MDA-MB-231 cells were seeded in 15 μ L at concentrations of 2000, 4000, or 8000 cells/well into A) Oris™ Pro 384 Tissue Culture Treated Plates or B) Oris™ Pro 384 Collagen I Coated plates. The number of cells that migrated into the exclusion zone was quantified at the indicated time points.

MDA-MB-231 cells are slow migrators and required 2 days to populate the exclusion zone. A high seeding density and collagen-coated plates were chosen for all subsequent assays.

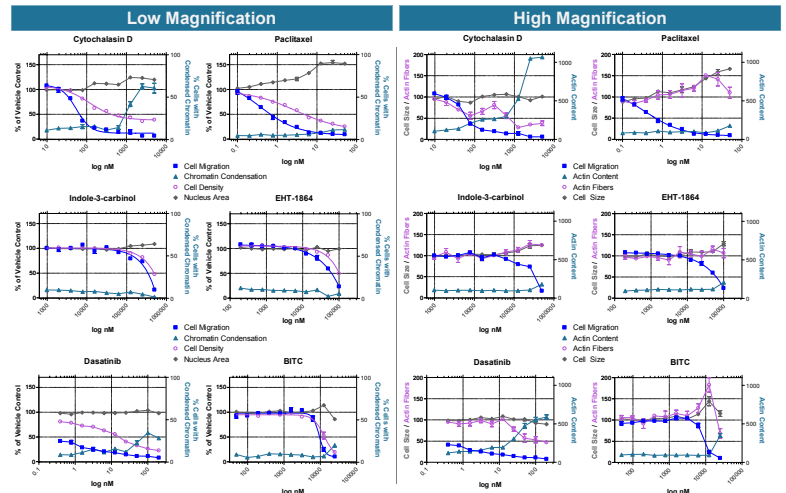
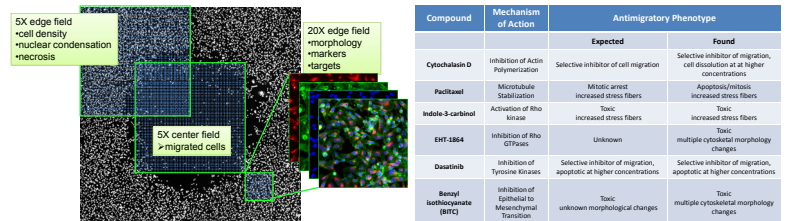
Assay Development: Automated Liquid Handling



MDA-MB-231 cells (10,000 in 15 μ L) were seeded into the wells of an Oris™ Pro 384 plate per condition and allowed to attach for 2 hr. Cells were fixed and stained with 4% formaldehyde containing Hoechst 33342 (10 μ g/mL) either immediately (pre-migration reference) or after 2 days incubation at 37°C. HTS assay performance parameters including Signal-to-Background (S/B), Coefficients of Variation (CV), and Z-factors were calculated for each experimental condition. Figure 1A shows the effect of Manual plating using an electronic multichannel pipette versus Automated cell seeding using the Titertek MAP-C2 bulk liquid dispenser. Figure 1B Demonstrates cell passage dependence by comparing cells from Passage 2 versus Passage 16.

The Oris™ Pro 384 assay delivered preliminary HTS performance parameters compatible with HTS requirements under manual or automated processing conditions. Assay performance was unaffected by cell passage in culture.

Multiparametric Profiling of Antimigratory Compounds



MDA-MB-231 cells were seeded into the wells of an Oris™ Pro 384 plate and treated after a 2 hr attachment period with 10-point, 2-fold dilution gradients of antimigratory agents with known mechanisms of action. After 48 hr, cells were stained with Hoechst 33342 and Cy3-conjugated phalloidin, and imaged at low (5x) and high (20x) magnifications on a Thermo Fisher Cellomics® ArrayScan®. Images were analyzed for cell migration, toxicity (cell density & apoptosis), cellular size, and cytoskeletal morphology (actin content and fiber count).

The Oris™ Pro 384 Cell Migration Assay permitted the simultaneous quantification of multiple, correlated parameters related to cell migration. Profiles for the different classes of agents were unique and consistent with literature reports.

Conclusions

The Oris™ Pro 384 Cell Migration Assay is a robust and convenient 2D cell migration assay that is compatible with HCS equipment for multiparametric analysis.

HTS Performance:

- The assay is compatible with automated liquid handling equipment for cell seeding, compound treatment, and sample processing.
- The assay satisfied universally accepted HTS performance criteria (Z-factors > 0.5)

Multiparameter High-Content Capabilities:

- The Oris™ Pro 384 Cell Migration Assay distinguished antimigratory agents by mechanism.
- Profiles for the different classes of agents were unique and consistent with literature reports.
- The information content of this assay can be further increased by including target markers of compound activity.

Acknowledgements

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The work is dedicated to Cameron Peterson, whose unexpected death prevented him from seeing the final results of his efforts.