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

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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-MB231 breast cancer cell line. This innovative cell migration assay utilizes a centrally located, self-dissolving, non-toxic biocompatible gel (BCG) to form uniformly sized, cell-free detection zones. Cells are seeded into 384-well clear bottom microplates that are compatible with a variety of high-content screening (HCS) platforms 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. The assay is logistically simple and does not require any additional processing steps, such as cell wounding or removal of physical barriers, which can be detrimental in cell migration studies. Using the number of cells that migrated into the exclusion zone as a primary readout, the assay was optimized for plating volume, cell seeding density and migration kinetics, 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 including inhibitors of tyrosine kinases, actin polymerization, mitosis and Rac GTPases. Upon compound treatment, cells exhibited dose-dependent loss of motility and assumed characteristic morphological phenotypes consistent with literature values. To translate these phenotypical observations into numerical values, digital images were acquired at two different magnifications on a ThermoScientific 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

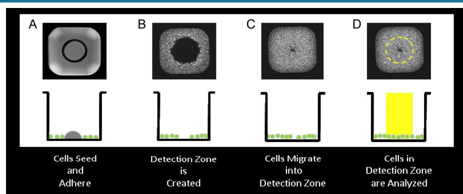
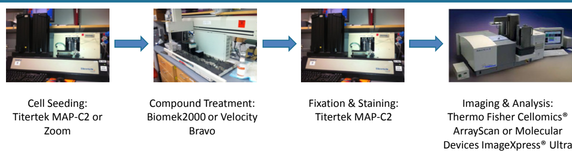


Figure 1: Schematic of Oris™ Pro 384 Cell Migration Assay.
A) Cells are seeded and adhere in an annular monolayer surrounding the Biocompatible Gel (BCG). B) BCG dissolves to reveal a cell-free Detection Zone. C) Cells migrate into the Detection Zone. D) Image cells via microscopy or High Content Imagers. Images are then analyzed for cell migration as well as phenotypical changes.

HTS Workflow



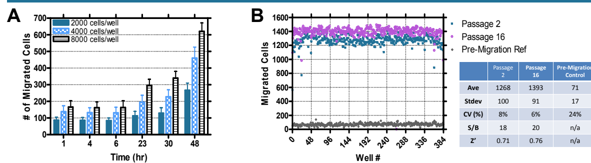
Cell Seeding: Titertek MAP-C2 or Zoom

Compound Treatment: Biomek2000 or Velocity Bravo

Fixation & Staining: Titertek MAP-C2

Imaging & Analysis: Thermo Fisher Cellomics® ArrayScan or Molecular Devices ImageXpress® Ultra

Assay Optimization



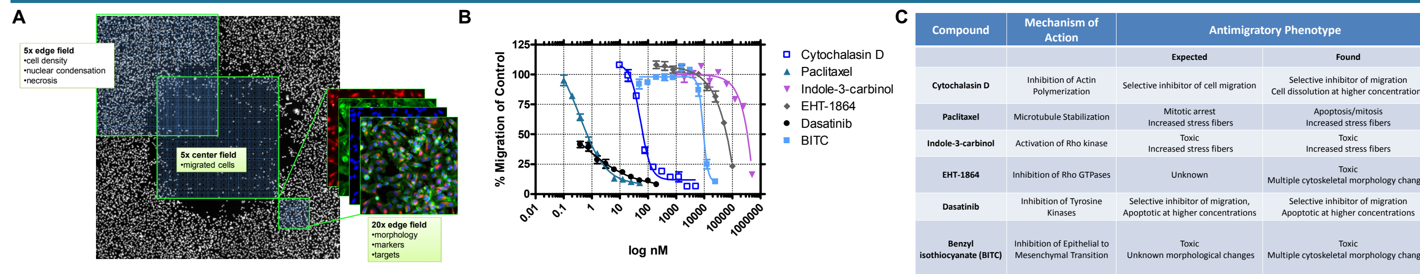
	Passage 2	Passage 16	Pre-Migration Control
Ave	1268	1393	71
Stdev	100	91	17
CV (%)	8%	6%	24%
S/B	18	20	n/a
Z'	0.71	0.76	n/a

Figure 2: Assay Optimization Cell Migration Assay with MDA-MB-231 cells.
MDA-MB-231 cells were seeded in 15 µL at concentrations of 2000, 4000, or 8000 cells/well into Oris™ Pro 384 Collagen I Coated plates (Figure 2A). The number of cells that migrated into the exclusion zone was quantified at the indicated time points. MDA-MB-231 cells (10,000 in 15 µL) from early and late passages were seeded into the wells of an Oris™ Pro 384 plate and allowed to attach for 2 hr (Figure 2B). Cells were fixed and stained with 4% formaldehyde containing Hoechst 33342 (10 µg/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.

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.

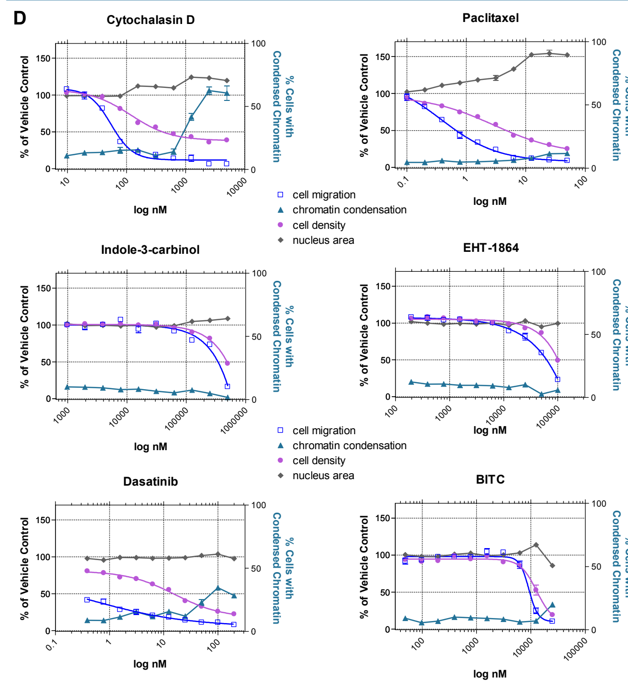
The Oris™ Pro 384 assay delivered preliminary HTS performance parameters compatible with HTS requirements and assay performance was unaffected by cell passage in culture.

Multiparametric Analysis of Antimigratory Compounds



Compound	Mechanism of Action	Antimigratory Phenotype	
		Expected	**Found**
Cytochalasin D	Inhibition of Actin Polymerization	Selective inhibitor of cell migration	Selective inhibitor of migration Cell dissolution at higher concentrations
Paclitaxel	Microtubule Stabilization	Mitotic arrest Increased stress fibers	Apoptosis/mitosis Increased stress fibers
Indole-3-carbinol	Activation of Rho kinase	Toxic Increased stress fibers	Toxic Increased stress fibers
EHT-1864	Inhibition of Rho GTPases	Unknown	Toxic Multiple cytoskeletal morphology changes
Dasatinib	Inhibition of Tyrosine Kinases	Selective inhibitor of migration, Apoptotic at higher concentrations	Selective inhibitor of migration Apoptotic at higher concentrations
Benzyl isothiocyanate (BITC)	Inhibition of Epithelial to Mesenchymal Transition	Toxic Unknown morphological changes	Toxic Multiple cytoskeletal morphology changes

Low Magnification



High Magnification

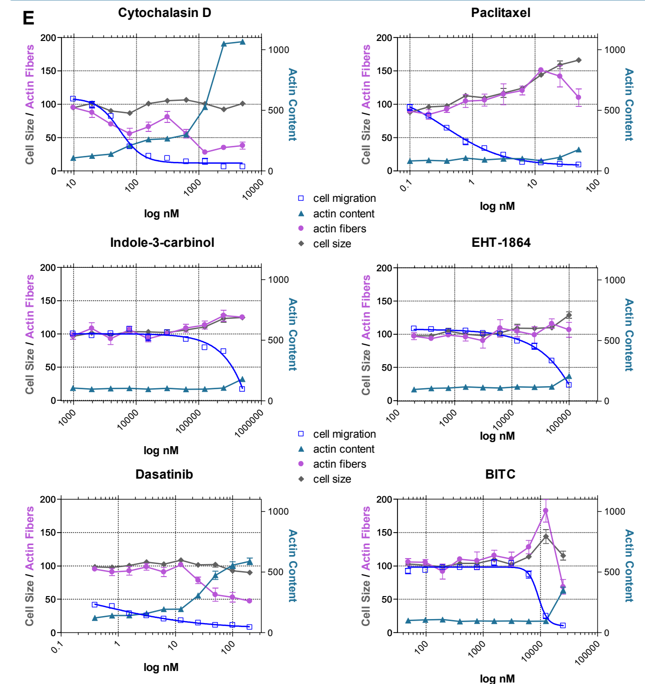


Figure 3: Multiparametric Analysis of Mechanistically Distinct Antimigratory Compounds
MDA-MB-231 cells were seeded into the wells of an Oris™ Pro 384 plate and treated after a 2 hr attachment period with 10-point, 2-fold serial dilutions of antimigratory agents with known mechanisms of action. After 48 hr, cells were stained with Hoechst 33342 and Cy3-conjugated phalloidin, and imaged (Figure 3A) at low (5x) and high (20x) magnifications on a Thermo Fisher Cellomics® ArrayScan®. 5x images of the center of the wells were used to determine inhibition of migration. 5x and 20x images taken on the outer edge of the wells were used to determine changes in cell health and morphology: toxicity (cell density & apoptosis), cellular size, and cytoskeletal morphology (actin content and fiber count). Cell migration dose dependent curves showed distinct differences in potency between compounds tested (Figure 3B). The 5x low magnification images were further analyzed for cell migration, chromatin condensation, cell density, and nucleus area (Figure 3D). The 20x high magnification images were further analyzed for actin content, actin fibers, and cell size (Figure 3E). The results of the multiparametric analysis were distinct across compounds and comparable to literature results (Figure 3C).

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

This work was partially supported by IMAT grant # 5R21CA147985-02 from the National Cancer Institute and grant # 2R44GM090386-02 from the National Institute of General Medical Sciences.

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