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 agents that inhibit cancer cell motility has been hindered by a dearth of high-throughput screening (HTS)-compatible cell motility assays. The **Oris™ Pro 384** well cell migration assay, developed by Platypus Technologies, is an innovative cell migration assay designed to enable HTS of potential anti-cancer compounds and wound healing agents on adherent tumor and endothelial cell lines. The assay utilizes a centrally located, non-toxic, biocompatible gel (BCG) to form a uniformly sized, cell-free detection zone into which cells migrate. The assay is logistically simple and does not require any mechanical processing steps, such as cell wounding or removal of physical barriers. The assay is fully compatible with laboratory automation, including robotic liquid handlers, plate washers, and high-content screening (HCS) readers.

A formal investigation of the robustness and HTS performance of the assay was conducted following guidelines in place at the University of Pittsburgh Drug Discovery Institute (UPDDI). Using MDA-MB-231 human breast cancer cells, the assay was optimized for migration kinetics, cell seeding density, and DMSO tolerance. In multi-day variability studies, the assay delivered signal-to-background ratios >10 and Z-factors above 0.5 on three consecutive days, was insensitive to process errors or edge-effects, and passed UPDDI Assay Protocol Approval Committee (APAC) criteria. The assay delivered equal HTS performance on two different HCS platforms – the Thermo Scientific Cellomics ArrayScan II and the Molecular Devices ImageXpress®Ultra point scanning confocal reader. Screening of a test cassette of 1280 compounds with known biological activities identified several agents with antimigratory activities. Simultaneous measurements of toxicity and cellular morphology permitted identification of agents that selectively inhibited cell migration in the absence of gross toxicity. All agents identified in the screen had targets associated with cell motility.

Our results validate the Oris™ Pro 384 cell migration assay as a logistically simple and information-rich cell motility analysis platform that meets the demands of large scale compound screening. The assay's compatibility with high-content imaging will greatly facilitate mechanism of action studies.

Assay Schematic

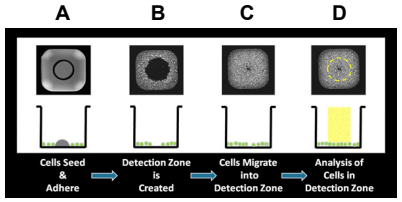


Figure 1: Schematic of Oris™ Pro 384 Cell Migration Assay.
A. Cells are seeded and allowed to adhere in an annular monolayer surrounding the Biocompatible Gel (BCG).
B. BCG dissolves to reveal a cell-free Detection Zone.
C. Cells migrate into the Detection Zone.
D. Cells are imaged via microscopy or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

HTS Workflow

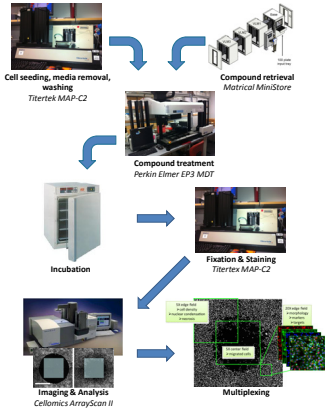


Figure 2: HTS Workflow for Laboratory Automation. The Oris™ Pro 384 Cell Migration Assay is compatible with automated liquid handling equipment. The HTS workflow was developed and implemented on laboratory automation equipment as shown: Titertek MAP-C2 bulk liquid handlers, Perkin Elmer EP3 MDT workstation for compound treatment, TC incubator and Cellomics ArrayScan II automated microscopy.

High-throughput Assay Development

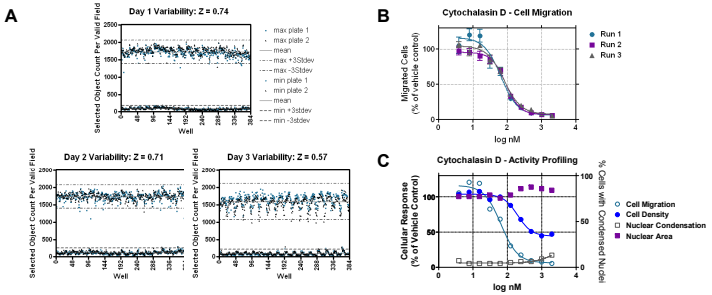
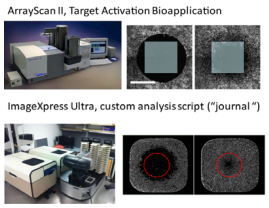


Figure 3: Multi-day Variability and Dose-response Reproducibility Studies. MDA-MB-231 cells were seeded at optimal density (15,000 cells/well) and fixed after a two-hour attachment (pre-migration, MIN control) or allowed to migrate for 2 days (MAX control). A single image in the center of the well was acquired on the ArrayScanII and cell numbers were enumerated. In multi-day variability studies using two Oris™ Pro 384 plates of maximum and minimum controls on three separate days, the assay delivered Z-factors above 0.5 on all 3 days. **A.** Scatter plots document that the assay performed well with full automation, with few process errors and tight distribution of positive and negative control samples. **B.** Dose response reproducibility: The control inhibitor, Cytochalasin D, gave essentially identical IC_{50} values of 65 nM, 90 nM, and 80 nM on three consecutive days. **C.** Multiparametric profiling of motility and toxicity, derived from single channel measurements of cell numbers and nuclear morphology, revealed that Cytochalasin D is a selective inhibitor of cell migration that slightly enlarges nuclei but does not cause apoptotic cell death or excessive cell loss.

Compatibility With Multiple Instruments

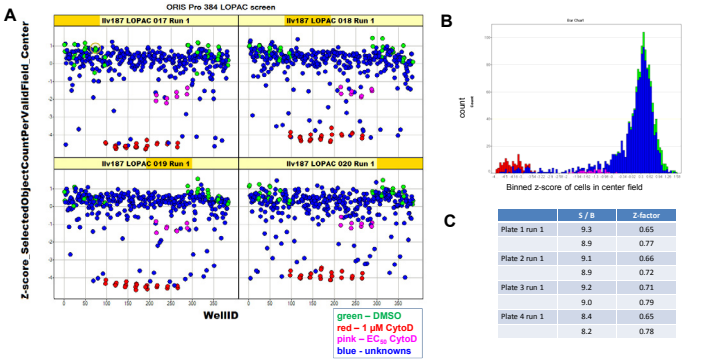


B

		ArrayScan	ImageXpress® Ultra
Day 1	CV min (%)	32.7	44.1
	CV max (%)	6.3	8.6
	S/B	19.1	26.7
	Z-factor	0.74	0.68
Day 2	CV min (%)	35.1	44.6
	CV max (%)	6.5	7.7
	S/B	14.1	31.7
	Z-factor	0.71	0.68
Day 3	CV min (%)	44.4	58.6
	CV max (%)	10.4	12.9
	S/B	17.2	25.0
	Z-factor	0.57	0.53
ALL DAYS	CV min (%)	37.4	49.1
	CV max (%)	7.7	9.7
	S/B	16.8	27.8
	Z-factor	0.67	0.63

Figure 4: The Oris™ Pro 384 Cell Migration Assay is Compatible with Multiple HCS Platforms. The Oris™ Pro 384 plates used in the three-day variability studies were read on the Cellomics ArrayScan II and the Molecular Devices ImageXpress® Ultra confocal point scanning HCS readers. **A.** Imaging strategies for both readers: On the ArrayScan II, a single image was acquired in the center of the well using a 5X objective. On the ImageXpress Ultra, the entire well was scanned using a 4X objective, and a custom MetaXpress journal was generated that detected migrated cells in a circular region of defined diameter. **B.** The assay delivered signal-to-background ratios > 10, Z-factors above 0.5, and low intra- and inter-plate variability on all 3 days. The assay delivered essentially equivalent results on both HCS platforms.

LOPAC Screen Using Oris™ Pro 384 Assay



	S / B	Z-factor
Plate 1 run 1	9.3	0.65
	8.9	0.77
Plate 2 run 1	9.1	0.66
	8.9	0.72
Plate 3 run 1	9.2	0.71
	9.0	0.79
Plate 4 run 1	8.4	0.65
	8.2	0.78

Figure 5: LOPAC Screening Using Oris™ Pro 384 Cell Migration Assay. Screening of the Library of Pharmacologically Active Compounds (LOPAC), a test cassette of 1280 compounds with known biological activities, identified several agents that inhibited cell migration on Oris™ Pro 384 plates. **A.** The screen consisted of four 384 well microplates in duplicate. Trellis plots from a single run are shown and depict z-scores, based on plate averages, of cells that migrated into the exclusion zone. Each microplate contained 32 wells of DMSO (green), 24 wells of 1 µM Cytochalasin D (red, positive controls), and 8 wells of 100 nM Cytochalasin D (pink, EC_{50} control), or unknown test compounds (blue). **B.** Distribution of screen data: Positive (red) and negative (green) controls were well separated. Unknowns (blue) largely followed a normal distribution. **C.** All plates had Z-factors in the range of 0.6 - 0.8 indicating HTS compatibility.

Multiparametric HCS Profiling of LOPAC

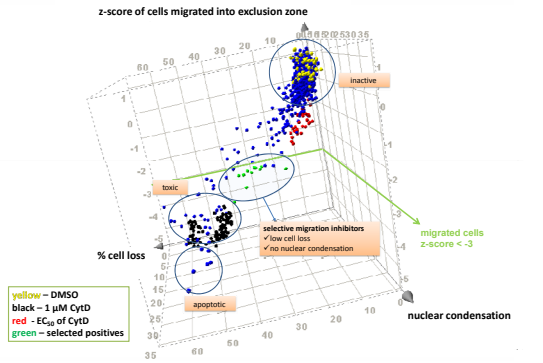


Figure 6: Multiparametric HCS profiling of LOPAC library using Oris™ Pro 384 Cell Migration Assay: Multiple phenotypic parameters were monitored during the primary LOPAC screen: Cell migration (y-axis), Cell loss (x-axis), and Nuclear Condensation (z-axis). The primary selection criterion (z-score of cells migrated into exclusion zone <-3) identified 47 antimigratory agents, a hit rate of 3.7% (green line illustrates cutoff). The multiparametric nature of the Oris™ Pro 384 Cell Migration Assay enabled separation of agents that caused cell loss ("toxic") and/or nuclear condensation ("apoptotic") from those that selectively inhibited cell migration in the absence of cellular toxicity ("selective migration inhibitors", marked in green). By these criteria, the screen identified nine compounds that selectively inhibited cell migration without being cytotoxic, for a final hit rate of 0.7%.

The Oris™ Assay Identified Compounds with Targets Known to Affect Cancer Cell Motility

Compound name	Targets	References linking targets to cell migration
DL-erythro-Dihydrosphingosine	PKC, PLA2, PLD	PKCs and PLD regulate tumor cell migration [1, 2]
GW5074	c-Raf1	Ras-MAPK pathway controls cell motility [3]
Bromoacetyl alprenolol menthane	beta adrenoreceptors, mTOR	mTOR mediates cell migration [4] Combination of Raf and mTOR inhibitors is synergistic in glioblastoma cell migration [5]
7-Cyclopentyl-5-(4-phenoxy)phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylamine	src-family kinases	src family kinases mediate cell migration [6]
Tyrphostin AG 879	TrkA, Her2	Receptor tyrosine kinases regulate cell migration [7]
GR 127935 hydrochloride	5-HT1B/1D receptors	Neurotransmitters are regulators of tumor cell migration [8] MDA-MB-231 cells express 5-HT receptors [9]
Indatraline hydrochloride	dopamine, norepinephrine and serotonin reuptake inhibitor	Neurotransmitters are regulators of tumor cell migration [8] MDA-MB-231 cells express 5-HT receptors [9]
3',4'-Dichlorobenzamil	Na+/Ca2+ exchanger	Na-channel knockdown inhibits glioma cell migration [10]
Dihydroouabain	Na+/K+ ATPase	Mammalian cardenolides in cancer prevention and therapeutics [11]

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Summary and Conclusions

The Oris™ Pro 384 Cell Migration Assay is a robust and convenient 2D cell migration assay that is compatible with robotic liquid handlers and HCS equipment for multi-parametric analysis.

In stringent multi-day variability assessment studies, the assay generated Z-factors > 0.5, signal-to-background ratios > 10, low intra-plate and inter-plate variability, and repeatable dose-response curves.

The Oris™ Pro 384 Cell Migration Assay was successfully employed to screen the LOPAC chemical library.

The multiparametric nature of the assay permitted the identification of compounds that selectively inhibited cell migration in the absence of overt cytotoxicity. All positives had molecular targets associated with cell migration.

The assay's compatibility with multiplexed imaging enables cellular mechanism of action studies.

Acknowledgements

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