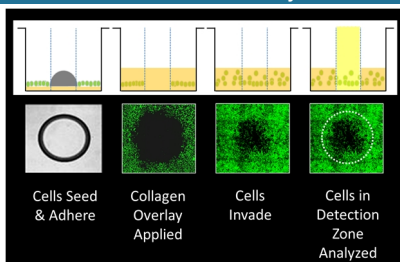


## Abstract

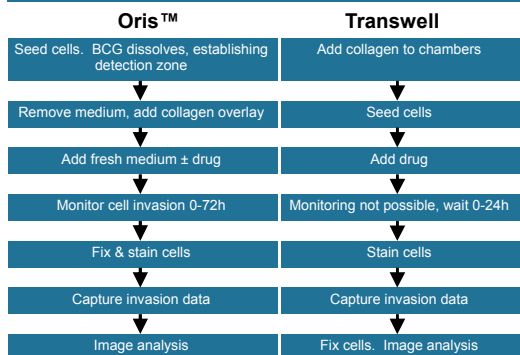
Cell invasion is the movement of cells through extracellular matrix (ECM). Invasion is of particular concern in cancer, where invading tumor cells lead to metastasis, the deadliest aspect of the disease. High-throughput assays suitable for screening the impact of candidate anti-cancer drugs on cell invasion are therefore highly sought after. Traditionally, cell invasion is quantified through "transwell" assays in which cells move from one chamber to another chamber through a synthetic membrane and ECM layer. However, transwell assays do not readily facilitate automation or real-time monitoring of invasion, nor simultaneous analysis of multiple cell phenotypes such as changes in morphology, nuclear structure, etc. Moreover, the use of a synthetic membrane to separate the two transwell chambers poorly simulates conditions found *in vivo*. Here we report development of an automatable cell invasion assay configured in an industry-standard 96-well format that can be used to monitor cell movement in three dimensions through ECM. The new assay seeds cells around an "exclusion zone". Following cell attachment, an overlay of collagen I is added. Cell movement into the exclusion zone is then monitored in real time, and cell morphology and physiology may also be monitored simultaneously. We report data comparing the information content and multiplex capabilities of traditional transwell invasion assays with the new exclusion zone invasion assay. We conclude that the fully automatable invasion assay facilitates major improvements in efficiency and information content over transwell assays, and promises to facilitate high content screening of cancer drug candidates for their potential to inhibit metastasis.

## Oris™ Pro 96-well Invasion Assay Overview



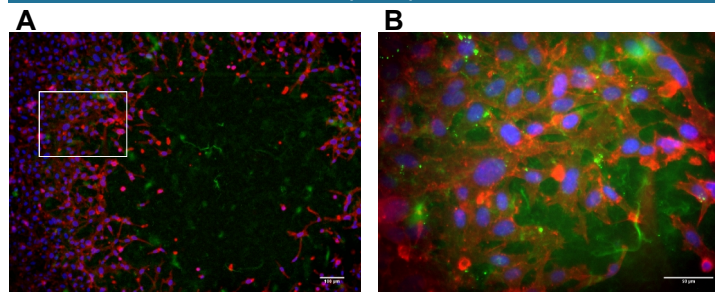
**Figure 1: Oris™ Pro 96-well Cell Invasion Assay.** Cells are seeded and allowed to adhere in an annular monolayer surrounding the Oris™ Pro Biocompatible Gel (BCG). Collagen I overlay matrix is added. Cells invade into the Detection Zone (DZ). Invasion is imaged and analyzed via fluorescence microscopy or High Content Imagers.

## Invasion Assay Workflow



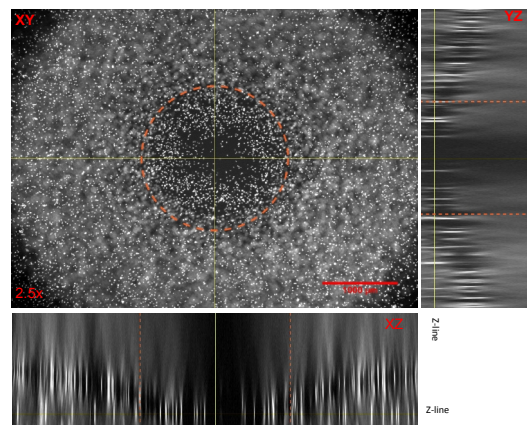
**Figure 2: Workflow for invasion assays.** Oris™: HT-1080 cells were seeded at 972 cells/mm<sup>2</sup> and allowed to adhere before removal of media. 40 µL Rat Tail Collagen Type I was overlaid at 2 mg/mL, followed by addition of fresh media ± drug. Cell invasion was assayed at multiple intervals by fixing with 0.25% glutaraldehyde, labeling with fluorescent stains, and imaged using a Zeiss Observer.Z1 fluorescence microscope. Transwell: Coated chambers with 9.5 µL Rat Tail Collagen Type I at 400 µg/mL. HT-1080 cells were seeded at 972 cells/mm<sup>2</sup> and media ± drug was added to wells. Cells were stained with Calcein AM and analyzed with a Bio-Tek Synergy HT fluorescence plate reader.

## Oris™ Multiplex Capabilities



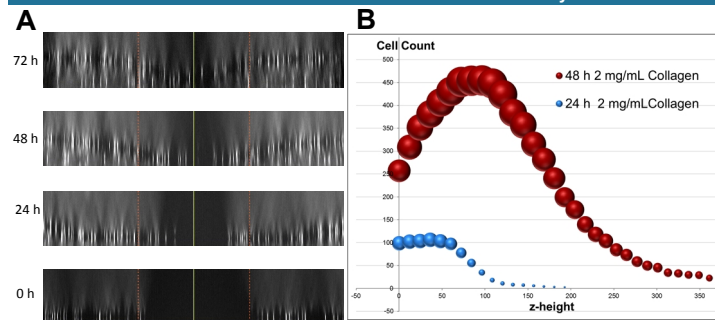
**Figure 3: Cells and collagen I overlay stained to examine morphology.** Cell invasion assay was performed with a 2 mg/mL collagen overlay and assayed at 24 h. DQ Collagen I Substrate (green) was added to the collagen overlay to monitor collagenase activity in real time. Cells were stained with TRITC-phalloidin (red) and DAPI (blue) at the end point. Images were captured with 10X (A) and 40X (B) objectives at their respective wavelengths.

## Oris™ Imaging Invasion and Analysis



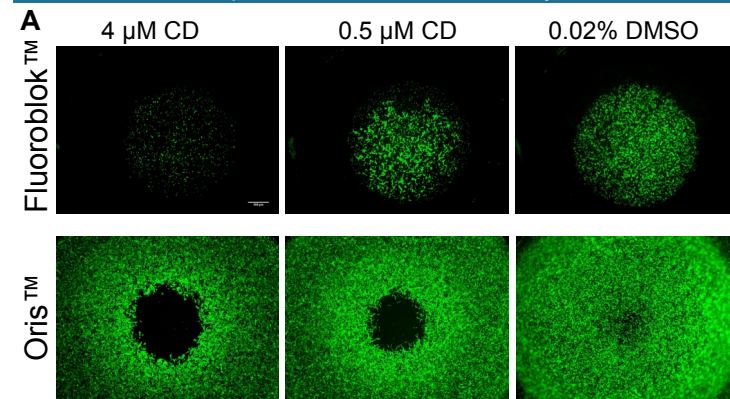
**Figure 4: Z-stack Image analysis using ImageJ to generate orthogonal views.** Above is a representative image taken with a 2.5x objective. Stacks of images were taken at optimal Nyquist rates of 21.39 µm and processed to obtain orthogonal views of the XZ plane (bottom panel) and YZ plane (right panel). The Z-lines were set at the zero plane for each well. Total height of Z-stacks are 1519 µm. The orange dashed lines superimposed onto all images, indicate the boundaries of the starting cell-free detection zones.

## Effect of Time Course of Oris™ Pro Invasion Assay



**Figure 5: Real-time monitoring of invasion in the Oris cell invasion assay.** Cell invasion assay was performed with a 2 mg/mL collagen overlay and assayed at 0, 24, 48, and 72 h. The results demonstrate an increase in cell invasion as a function of time. (A) Orthogonal views demonstrate cell movement into the DZ (orange lines). At 48 h significant invasion into the Z-plane and DZ was observed with 2mg/mL collagen overlay. (B) Quantification of invasion by MetaMorph® research imaging software (Molecular Devices) confirms visual observation. An increase in invasion is observed over time at different Z heights (0-373 µm).

## Comparison of Oris™ versus Transwell Assay



**Figure 6: Inhibition of invasion with Cytochalasin D.** Cell movement of HT-1080 cells with Fluoroblok™ and Oris™ Pro platforms was assayed at 26 and 46 hours respectively. Plates were analyzed using fluorescence spectrophotometry and fluorescence microscopy. (A) Representative images of indicated concentrations of Cytochalasin D and Invasion Control (0.02% DMSO). (B) Results demonstrate dose dependent inhibition of invasion by Cytochalasin D with IC50 values of 0.4148 µM (Fluoroblok™) and 0.3918 µM (Oris™). (C) Graph of Invasion Control results. Z' values were 0.50 for Fluoroblok™ and 0.89 for Oris™.

## Summary and Conclusions

Assay conditions for the Oris™ Pro 96-well Cell Invasion Assay can be optimized by varying collagen concentration (data not shown) & assay duration.

The degree of 3-D invasion is dependent on concentration of collagen in the overlay (data not shown) and is time dependent.

Images of invading cells in the Z-plane can be captured by fluorescence microscopy and analyzed using ImageJ (NIH).

Orthogonal view images demonstrate the extent of cell invasion into the Z-plane.

Cell motility is inhibited using Cytochalasin D in both the Oris™ and transwell formats.

Data generated with Oris™ had smaller standard deviations and higher Z' values than with transwell.

The Oris™ Pro 96-well Invasion Assay is versatile, allowing convenient 3-D cell invasion analysis with unrestricted access, measurement of cellular invasion in the Z-plane, and compatibility with automation and HCS.

## Acknowledgements

This work was partially supported by grant 2R44GM090386-02 from the National Institute of General Medical Sciences (NIH) www.platypustech.com info@platypustech.com

