High Content Analysis of an Automatable 3-Dimensional Cell Invasion Assay

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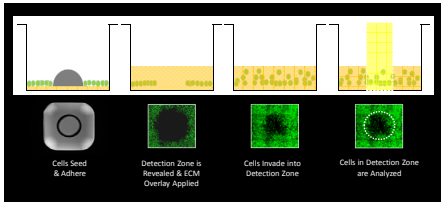
Abstract

The ability of cells to invade through an extracellular matrix is a hallmark event in the metastasis of tumor cells. Understanding the signaling pathways involved in invasion is crucial for discovering new targets to develop anti-metastatic drugs for treating cancer. Because cell invasion is a phenotypic or whole-cell event, it can be challenging to conduct and quantitatively analyze such cell-based assays in a screening format. A novel invasion assay platform that is amenable to automated liquid handling now allows for both an increased capacity and decreased hands-on time for screening assays. This assay utilizes a centrally located self-dissolving biocompatible gel (BCG) to form a uniformly sized, cell-free detection zone on a collagen I coated cell culture surface. Cells are seeded into 96-well plates and pattern in an annular monolayer surrounding the BCG. Once the BCG dissolves, an overlay of collagen I is applied to the assay wells and cells can invade in 3-dimensions into the detection zone previously occupied by the BCG. Inhibitors, such as Cytochalasin D, may be added to the assay wells in the media covering the collagen I overlay. This assay format allows visual assessment of cell invasion throughout the duration of the experiment. Cells may be fixed and treated with multiple stains, including DAPI to visualize nuclei and TRITC-phalloidin to observe F-actin. Such stains enable flexible data capture by either enumerating invading cells or by calculating the area of closure within the detection zone. Here we demonstrate successful analysis of the Platypus Technologies Oris™ Pro Collagen I Cell Invasion Assay using the Molecular Devices ImageXpress® Micro Widefield HCS System and MetaXpress® and AcuityXpress™ Software and demonstrate robust and reproducible quantification of HT-1080 cell invasion in 3-dimensions.

Assay Schematic



Methods

• HT-1080 cells were seeded at 30,000 cells/well into wells of an Oris™ Pro Collagen I coated 96-well assay plate.

• After 1 h, seeding medium was removed and 40 µL of a 3 mg/mL Collagen I overlay was added to assay wells and allowed to polymerize for 1 h before the addition of medium containing Cytochalasin D or vehicle only.

• The cells were incubated for 72 h to permit invasion followed by fixation with 0.25% glutaraldehyde and dual labeling with DAPI and TRITC-phalloidin.

• Images were acquired on the ImageXpress® Micro at 10X magnification using DAPI excitation and emission filters. Sixteen sites per well were captured, covering the entire 2 mm invasion zone. At each site, z-series images were acquired over a 120 µm range in 40 µm steps. Using MetaXpress® image acquisition and analysis software, the sites in each z-plane were montaged into a single image and the montaged images were combined in a z-stack. MetaXpress® tools were then used to measure the empty volume of the migration zone and to count the cells in the migration zone at each z-plane. AcuityXpress® software was used to calculate IC_{50} values.

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3-Dimensional Visualization of Invading Cells

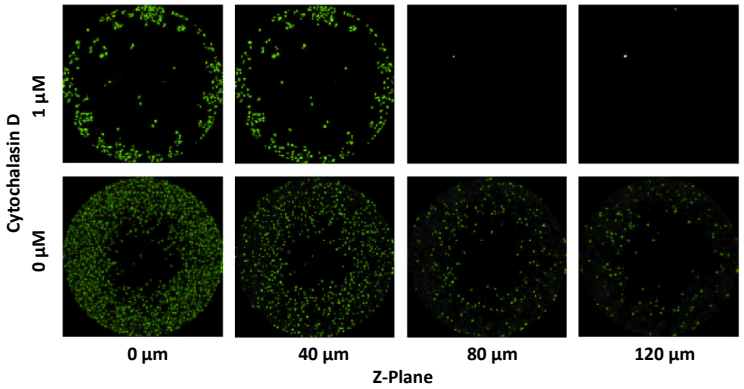

Figure 1. Visualization of nuclei in the detection zone of each z-plane. Images of DAPI stained cells (pseudocolored green) were captured in a z-series with 40 µm steps over a 120 µm range using the ImageXpress® Micro. At each z-plane, a montage of images from 16 sites was created, the detection zone was isolated, and the number of cells was counted.

In the absence of Cytochalasin D, HT-1080 cells invaded 120 µm into the z-plane of the Collagen I overlay. Treatment of cells with 1 µM Cytochalasin D dramatically reduced invasion into the Collagen I overlay.

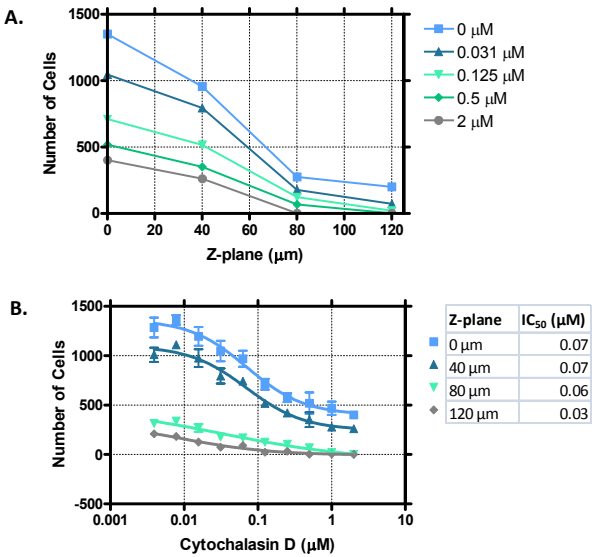

Figure 2. Effect of Cytochalasin D on invasion. A) Number of cells per z-plane for each concentration of Cytochalasin D tested. B) Dose response of Cytochalasin D in each z-plane.

Cytochalasin D inhibited the invasion of HT-1080 cells into the collagen I overlay in a concentration-dependent manner.

Effect of Cytochalasin D on 3-Dimensional Cell Invasion

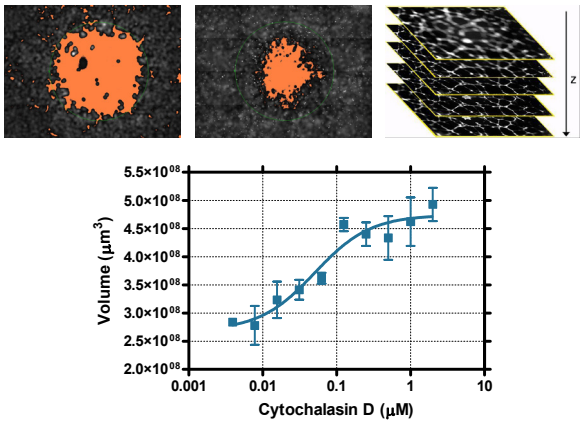

Figure 4. Volumetric changes to the detection zone by invading HT-1080 cells. Montaged images from each z-plane were assembled into a z-series and the empty volume of the detection zone was calculated using MetaXpress® software. Representative composite images created by montaging 16 sites captured in each z-plane are depicted for Cytochalasin D treated cells (***left image***) and cells treated with 0.1% DMSO vehicle only (***middle image***). At each of the 4 focal planes between 0 and 120 µm, the empty area was thresholded (depicted in orange) and the 3 dimensional volume was extrapolated from the area measurements and the z-distance between planes (***right image***). The graph depicts the dose-dependent effect of Cytochalasin D on the volume of the open area of the detection zone to a height of 120 µm into the Collagen I overlay.

Using a 2µM concentration of Cytochalasin D as a pre-invasion reference, the initial volume of the detection zone to a height of 120 µm was calculated to be 4.9 x 10^8 µm³. In the absence of Cytochalasin D, the cell invasion results in a measured volume of 2.8 x 10^8 µm³, corresponding to a 43% reduction from the initial volume.

Cytochalasin D arrested the invasion of HT-1080 cells into the 3-dimensional Collagen I overlay with an IC_{50} of 0.07 µM.

Conclusions

Oris™ Pro Cell Invasion Assays are easy to use, robust and fully automatable while providing real-time visibility of cell movement.

Oris™ Pro Cell-based Assays are attractive options for High Throughput Screening and High Content Analysis of modulators of cell motility.

The ImageXpress® Micro coupled with MetaXpress® and AcuityXpress® software provides flexible image capture and analysis of cells in the Detection Zone in the x, y and z-axes.

Acknowledgements

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