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.

**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 10× 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 combined into a single image and the montage 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 IC₅₀ values.

**Results**

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.

**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.