A Fully Three-dimensional Assay for High-content Analysis of Cell Invasion

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Abstract

Cell invasion is the movement of cells through the 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 needed.

Traditionally, cell invasion assays are performed by seeding cells in an upper compartment and assessing invasion by counting cells that penetrate through a flat, two-dimensional (2D) ECM-coated porous membrane into a lower chamber. However, the membrane is unlike any in vivo structure, potentially compromising physiological relevance of the assay. Moreover, traditional invasion assays preclude real-time monitoring, as cells can be viewed microscopically only after their journey through the membrane, precluding real-time analysis of changes in migration rate and morphology.

In this work, a fully three-dimensional (3D) assay was utilized where cells were embedded in a collagen type I matrix through the entire assay to mimic more closely the in vivo microenvironment. Cell invasion was monitored via “exclusion zone” technology. This assay was performed in industry-standard 96-well plates compatible with full automation and with high content imaging. Briefly, physical stoppers were inserted in wells of a 96-well plate, and HT1080, MDA-MB-231, or HaCaT cells were mixed with collagen and seeded around stoppers. After matrix polymerization, the stoppers were removed, creating a cell-free “exclusion zone” in the centers of each well. This central zone was then filled with fresh collagen.

3D cell invasion was monitored from the surroundings into the central zone. No cell tracking was needed to observe cells that invade into this zone. After 6-7 days of culture, 3D cell distribution was evaluated in the central zone between 0-800 µm heights, confirming the 3D nature of the assay. Migration/invasion inhibitors Cytochalasin D, Latrunculin A, and GM6001 effectively inhibited 3D invasion of HT1080 and MDA-MB-231 cells, whereas epidermal growth factor stimulated MDA-MB-231 3D cell invasion. Real time invasion studies were performed and different invasion rates were found for the three cell lines.

These results establish a novel, efficient, and information-rich assay that promises to improve high content screening of drug candidates in cancer research.

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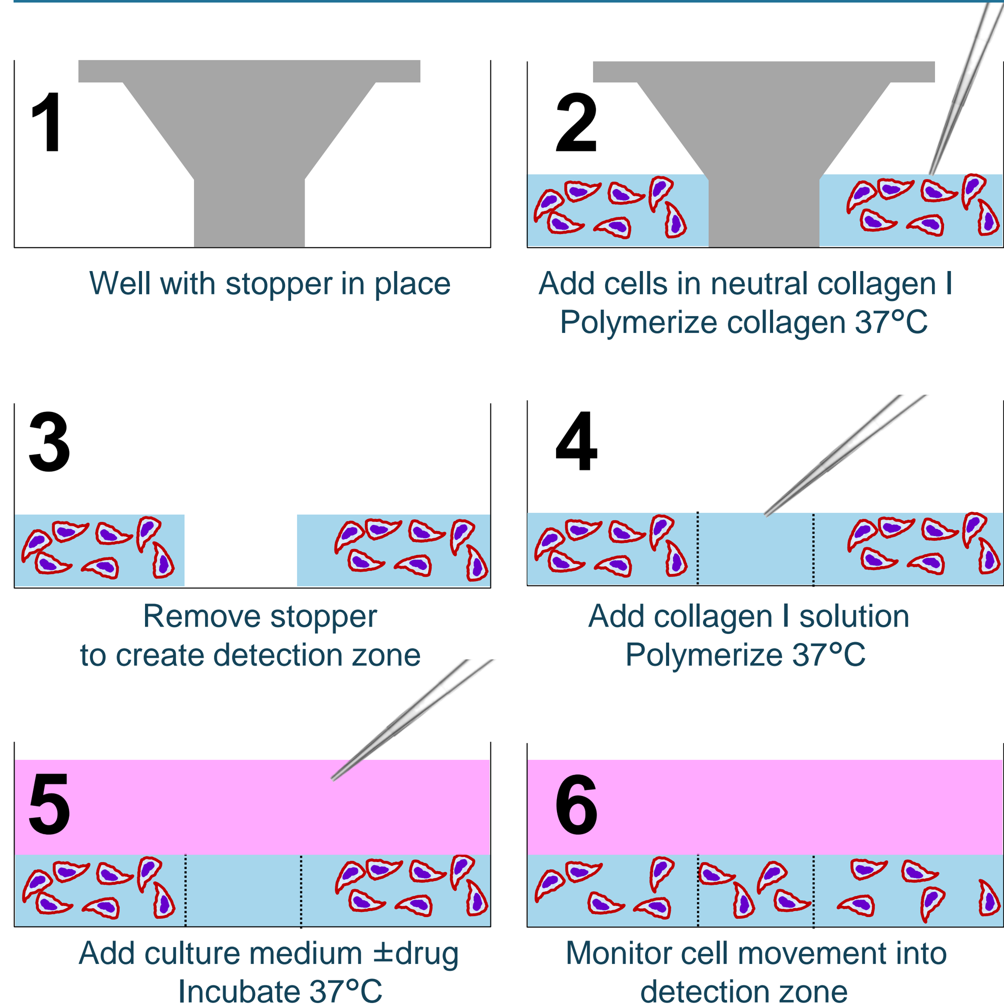
Assay Overview



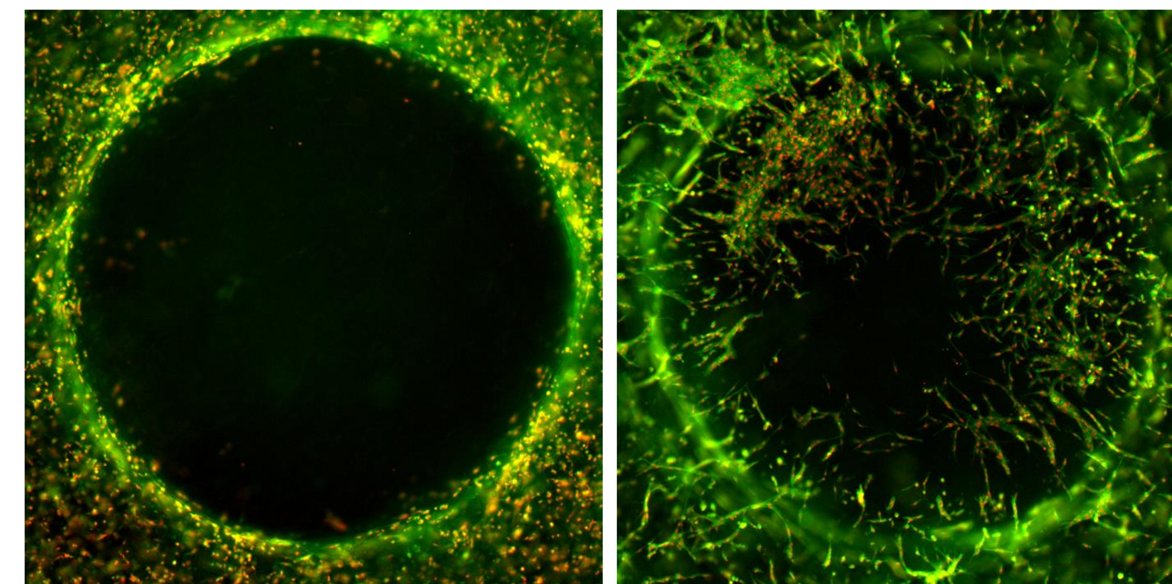
The Oris™ 3D Embedded Invasion Assay plates are provided with a Collagen I coating and with stoppers that fit tightly in each well of a 96-well plate.

Cells are seed in a thick layer of collagen I around the stoppers. When the collagen is polymerized, stoppers are removed and the central column is filled with collagen I to form the detection zone. Movement of cells through the detection zone is then assessed microscopically or using plate readers.

For quantification by microscopy, cells in the detection zone are counted in multiple focal planes. Counts may be summed for each well or plotted separately for each plane.

For quantification by plate readers, cells may be stained, and fluorescence intensity in the detection zone measured in conjunction with a physical mask.

96-Well Exclusion Zone Technology



Pre-Invasion   **Post-Invasion**

In the 3D Embedded Invasion Assay, cells are suspended in a thick layer of collagen I surrounding a 2-mm circular central cell-free collagen I detection zone (left image). After several days incubation, invading cells move into the detection zone (right image) where they can be unambiguously quantified. Images are false-color composites of the detection zone stained for nuclei (red) and actin (green).

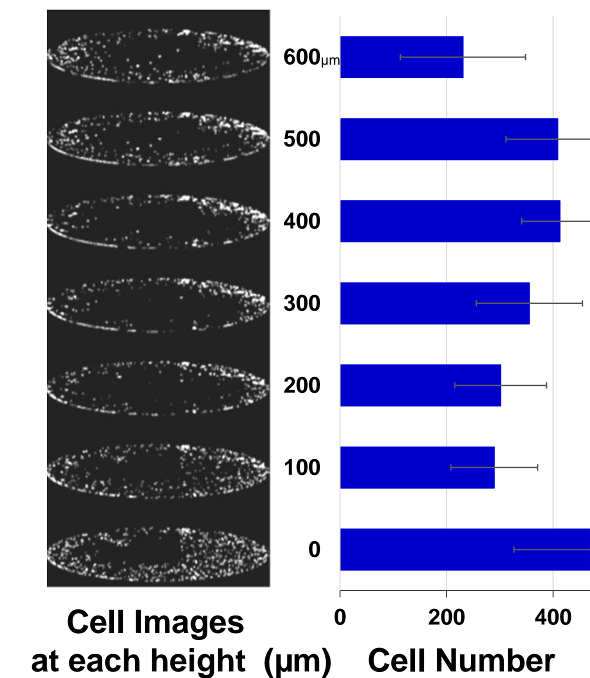
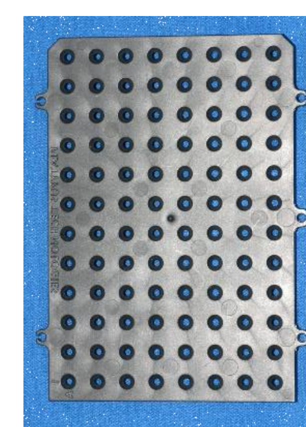
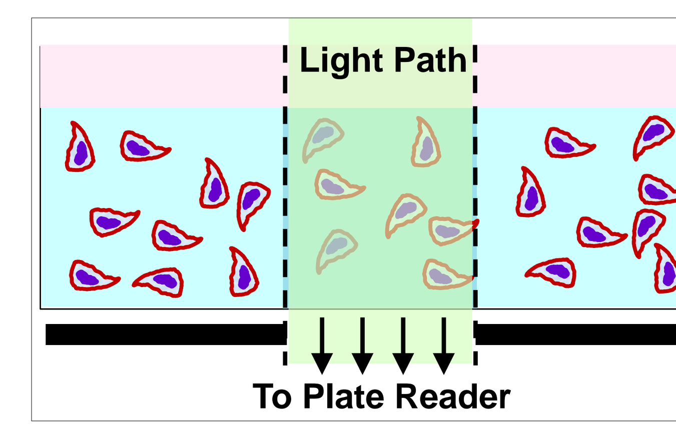


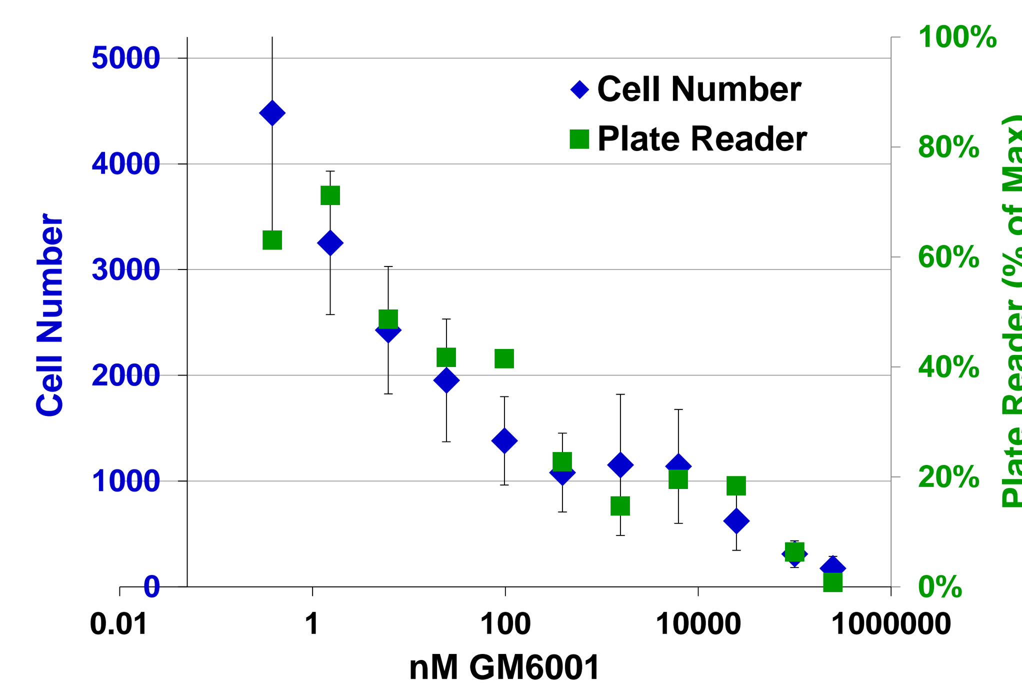
Image analysis (left) of the embedded cells shows cells in all vertical planes, and distribution of cell number (right, histogram) confirms uniform 3D distribution of cells.

Plate Reader vs Image Analysis



Left: The Oris Mask with 8 x 12 apertures clips to the bottom of the 96-well plate, masking all except the detection zone of each well.
Right: Side view of one well showing light path through the mask aperture, facilitating quantification of cell numbers using plate readers for fastest data acquisition.

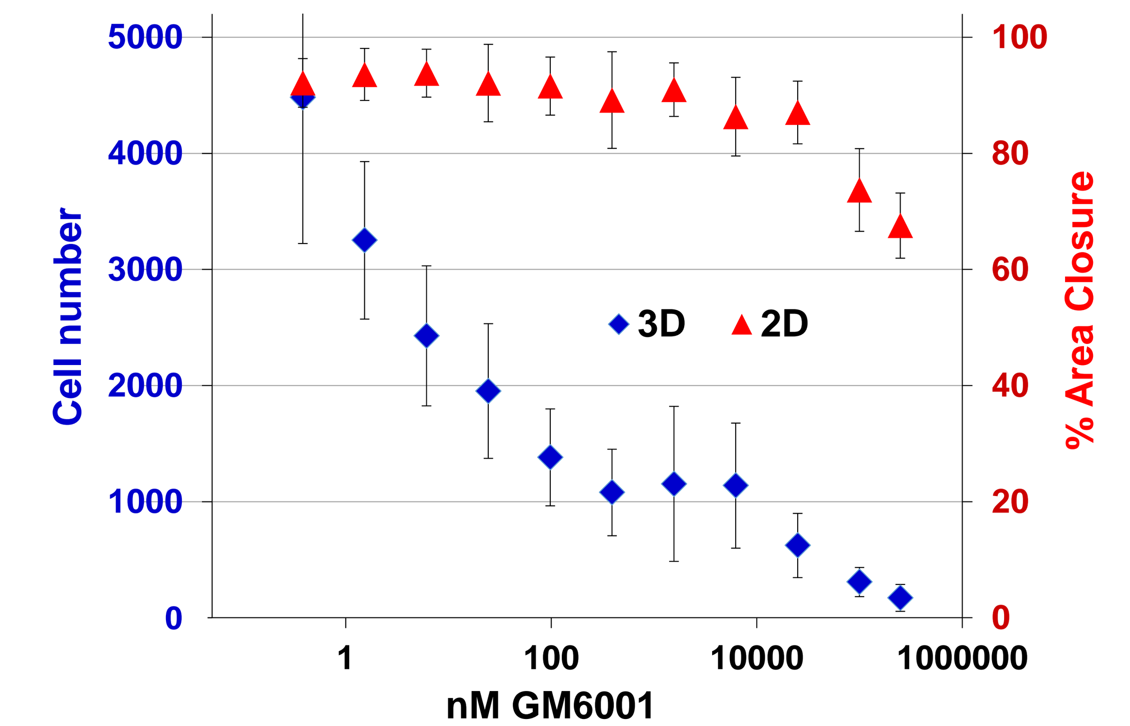




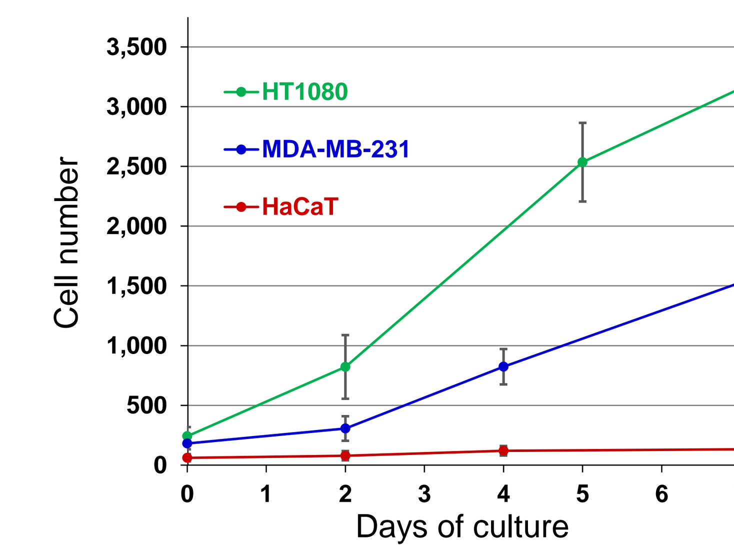
30,000 HT1080 cells were seeded per well in the Oris 3D Embedded Invasion Assay. Culture medium ± GM6001 was added after collagen in the exclusion zone polymerized. After 7 days incubation, cells were fixed and stained with DAPI. 3D invasion was quantified (i) by image analysis of cells invading into the exclusion zone (blue data points) and (ii) using the mask and a plate reader to measure fluorescence intensity in the exclusion zone (green data points). All data points average of six wells ± sd.

Differential Inhibition of 3D Invasion

Oris 3D Embedded Invasion Assay (3D, blue) and Oris Cell Migration Assay (2D, red). 30,000 HT1080 cells/well were treated with culture medium +/- GM6001. After 1 day (2D) or 7 days (3D), cells were fixed and stained with DAPI. Quantification of captured images was performed in 3D invasion by counting cells in the exclusion zone. 2D migration was quantified by measuring percent area closure in the exclusion zone. All data points average of six wells ± sd.



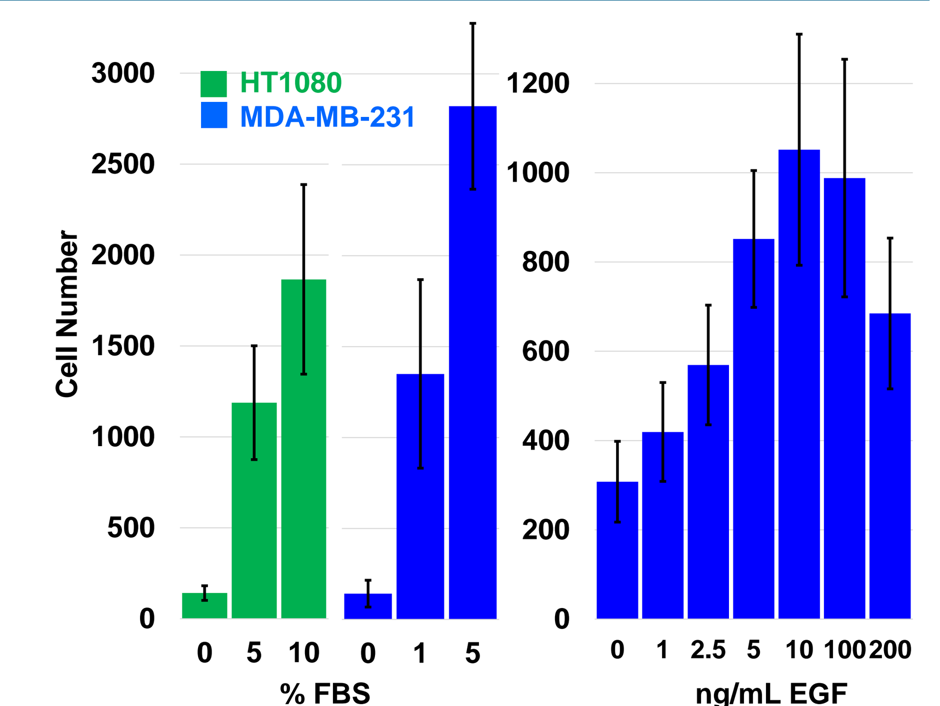
Comparing Cell Invasion Rates



Time course of invasion for three cell types seeded at 50,000 cells per well in the Oris 3D Embedded Invasion Assay. Human HT1080 fibrosarcoma and MDA-MB-231 breast adenocarcinoma cell lines are known to be invasive. Assay results show that these two cell types invade through collagen I at distinct rates. Control, noninvasive HaCaT keratinocytes invaded little, validating the assay for assessment of invasive cell behavior.

Stimulation of Invasion by FBS & EGF

Serum starved cells were treated with fetal bovine serum (FBS) in the medium in the Oris 3D Embedded Invasion Assay. After incubation (HT1080: 6 days, MDA-MB-231: 14 days), there was dose dependent stimulation of invasion by FBS for both cell types. Serum starved MDA-MB-231 cells were also incubated with epidermal growth factor (EGF) in both the medium and collagen gel. Stimulation of invasion by EGF was assessed after 7 days of incubation.



Conclusions

- The Oris 3D Embedded Invasion Assay can be used to monitor cell invasion entirely in 3D from cell seeding through to fixation
- The assay supports real-time multiparametric monitoring; no inserts or membranes occlude continuous microscopic observation
- Invasion can be quantified using image analysis or plate readers; no elaborate cell tracking system is needed
- The assay identifies specific inhibitors & stimulators of invasion, and distinguishes impacts of drugs on 3D invasion vs 2D migration

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