Investigation of Cell Migration in a Label-free Cell Exclusion Assay by Automated Microplate Imaging

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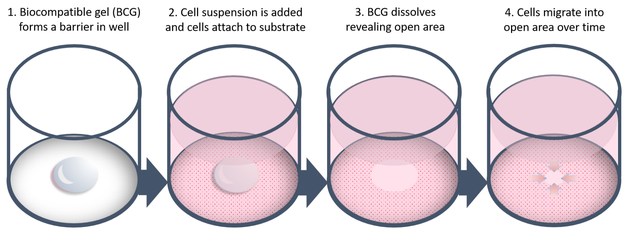
Introduction

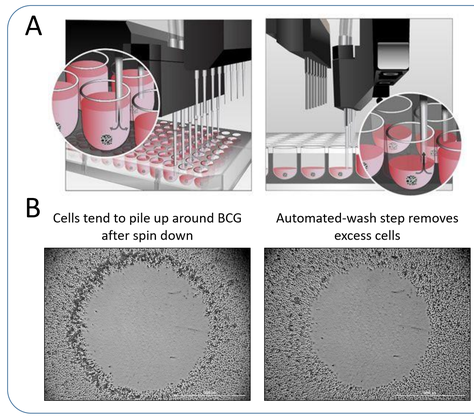
- Cell migration is the movement of cells from one location to another, driven by complex environmental signaling events and induced structural changes to the cytoskeleton.
- Multicellular organisms rely on cell migration for a vast array of biological processes, including embryonic development, immune responses, wound healing, and cancer metastasis.
- The need to better understand the mechanisms that regulate cell migration has led to the development of improved methods of investigation, including specialized cell culturing platforms and live-cell imaging techniques.
- Here we demonstrate an automated kinetic imaging-based approach to investigating cell migration using the Oris™ Cell Migration Assay Kits.
- This convenient label-free method enables accurate and robust analysis of cell migration within a 96- or 384-well platform for high-throughput applications.

Oris™ Pro Cell Migration Assay



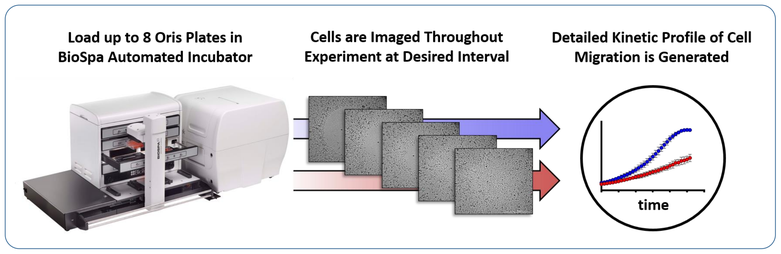
Oris™ Pro assays use a water-soluble biocompatible gel (BCG) to create a central cell-free detection zone in the center of each well of a 96- or 384-well microplate. Cells are seeded directly in the untreated or extracellular matrix (ECM)-containing wells manually or with an automated liquid handler. Once the BCG dissolves (within 20 minutes), the cell free zone is revealed, allowing cells to migrate into the open area.

Automated liquid handling increases efficiency and assay performance



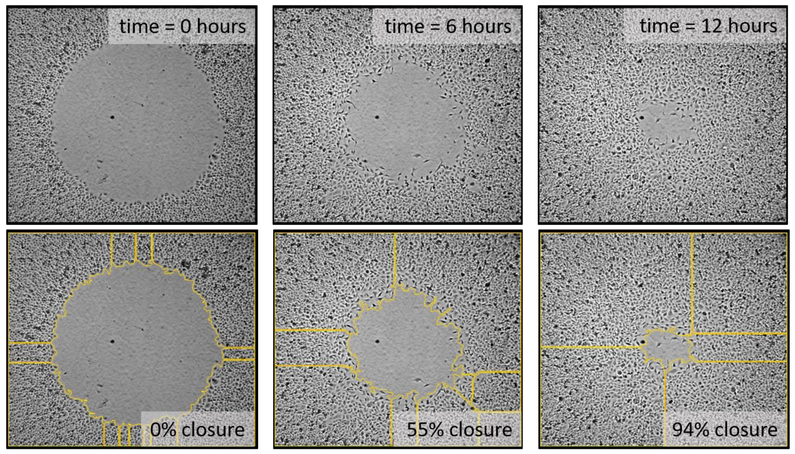
Automated cell dispensing (100 µL in 96-well and 20 µL in 384-well) was carried out using a MultiFlow FX Multi-Mode Dispenser. After a brief spin to settle cells, plates were kept at room temperature for 20 minutes to allow to fully attach to the substrate. (A) Before adding test compounds, three wash cycles with media were performed using the MultiFlow FX to (B) remove excess cells that tend to collect along the rim of the open area. This step ensures even cell density and improves accuracy and reproducibility of results.

Kinetic live cell imaging workflow



Prepared Oris™ plates were loaded into the BioSpa 8 Automated Incubator which maintained optimal growth conditions (37° C, 5% CO_2, and 80% humidity) throughout the experiment. Upon starting the cell migration imaging protocol, each plate was delivered to the linked Cytation 5 Cell Imaging Multi-Mode Reader every hour. Image analysis settings were automatically applied, generating quantitative migration results in real-time.

Automated image analysis of cell migration

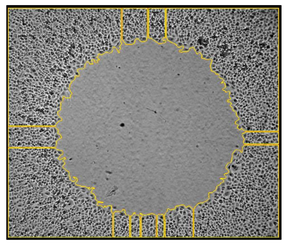


Automated image analysis is conducted by Gen5 software in real-time to determine the total image area occupied by cells (outlined in yellow) throughout the experiment. Percent closure (open area relative to starting open area) is then automatically calculated and reported.

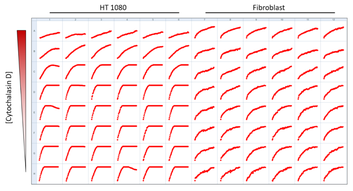
Comparison of results from 96- and 384-well formats

96-well format

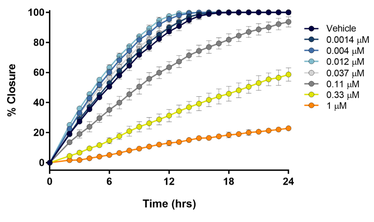
Images captured using 2.5x objective



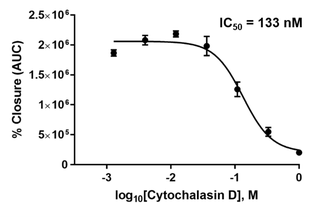
96-well matrix view of % closure profiles testing serial dilutions of Cytochalasin D



Mean % closure profile for HT1080 at each Cytochalasin D concentration

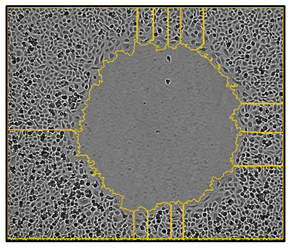


HT1080 dose response calculated using area under the curve (AUC) for each kinetic profile

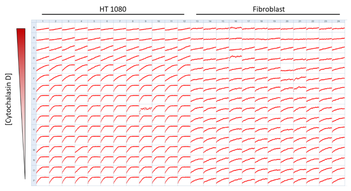


384-well format

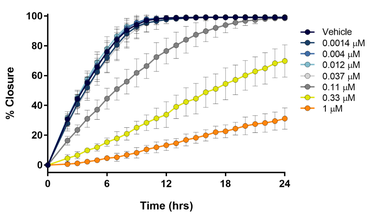
Images captured using 4x objective



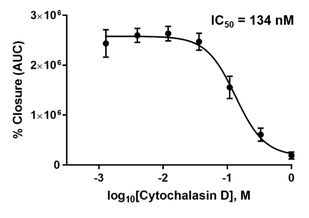
384-well matrix view of % closure profiles testing serial dilutions of Cytochalasin D



Mean % closure profile for HT1080 at each Cytochalasin D concentration

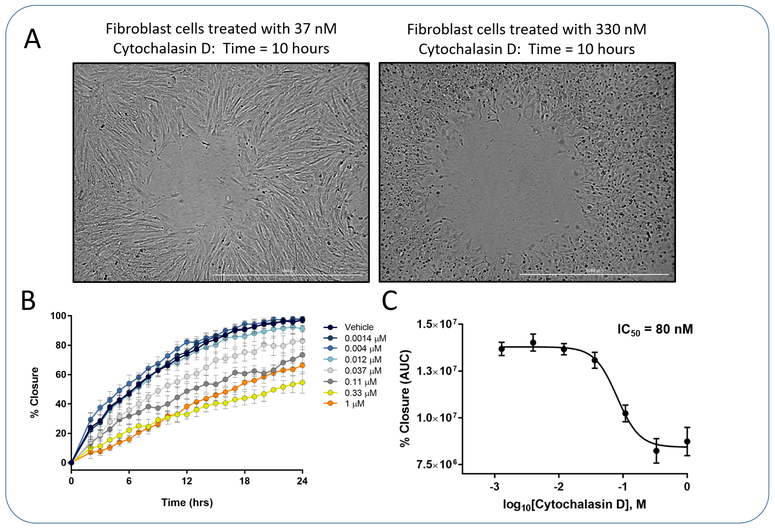


HT1080 dose response calculated using area under the curve (AUC) for each kinetic profile



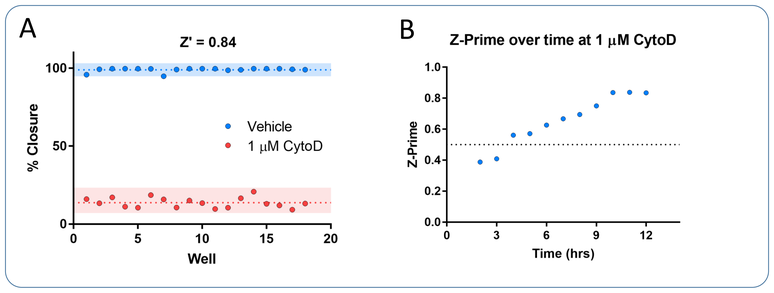
The effects of Cytochalasin D on HT1080 and human fibroblast migration were evaluated using the Oris™ Pro 96- and 384-well formats. Each well in the 96-well plate was imaged using the 2.5x objective, while the 384-well protocol used the 4x objective. Both the 96- and the 384-well formats generated highly consistent results across replicates. The smaller size of the initial open areas in the 384-well plates resulted in slightly faster rates of closure compared to the 96-well. However, the relative migration rates, and IC_{50} values calculated from the AUC of the kinetic profiles, were nearly identical.

Detailed images enable qualitative evaluation of cell health and morphology



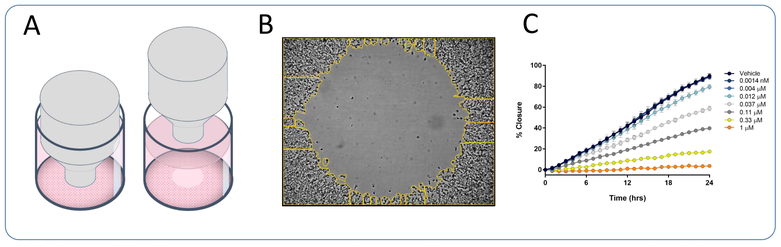
(A) High resolution images provide a detailed qualitative record of cell health and morphology that can be used in combination with (B,C) quantitative results to further evaluate treatment effects. Fibroblasts treated with 37 nM Cytochalasin D had significantly inhibited migration rates compared to untreated cells without appreciable changes in morphology. In contrast, cells treated with 330 nM Cytochalasin D exhibited altered morphology and apparent cell death.

Robust platform for high-throughput screening studies



Reproducible results generate a high Z-prime (Z'= 0.84 and 0.81 with the 384- and 96-well format, respectively). (A) Results from 384-well format at 1 µM CytoD at time=12 hours. (B) Z' values over the first 12 hours of the assay. Accuracy and sensitivity of assay delivers high Z' within a short amount of time (Z'= 0.56 at time=4 hours, 1 µM CytoD).

Compatible with Oris™ Cell Migration Assay "Stoppers"



(A)The Oris™ Cell Migration Assay uses "stopper" barriers to create a central cell-free detection zone in the center of each well of a 96-well plate. Although this platform is not compatible with automated cell seeding, it's designed for maximum flexibility in assay design, including custom ECM applications and cells requiring a long adhesion period. (B) Automated image capture and analysis produces results comparable to the Oris™ Pro platform. (C) Kinetic cell migration profile of Cytochalasin D-treated HT1080 on a tissue culture treated 96-well plate.

Conclusions

- This label-free, fully automated method provides a convenient and accurate solution for measuring cell migration using the Oris™ cell exclusion platforms.
- Automated liquid handling steps increase efficiency and improve assay results.
- Gen5 image analysis software delivers reproducible quantitative results using both the 96- and 384-well formats, while capturing a detailed qualitative record of cell morphology.
- High Z' value for high-throughput screening applications.