

High Throughput Imaging Assay to Assess Cell Migration

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Abstract

Assays that measure cell migration are critical for studying the mechanisms involved in diseases such as metastatic cancer, inflammation, and wound healing or chronic sores. Here we report on the use of the IsoCyte Dual Laser Scanner and ImageXpress® Micro Imaging Systems combined with the Oris™ and Oris™ Pro Cell Migration Assays (Platypus Technologies). The kits consist of a 96-well plate where cell monolayers are formed in the presence of either a silicone stopper or a dissolvable biocompatible gel that blocks cells from seeding in the center of each well. After the stoppers are removed or the gel dissolves, cell migration into the central detection zone is measured in fluorescently labeled live or fixed cells. The IsoCyte and ImageXpress Micro platforms can scan whole well areas making them ideal instruments for rapidly acquiring cell migration images within the detection zone in each well. The IsoCyte instrument can analyze the images as they are acquired (on-the-fly) thus providing raw images as well as total area and intensity data within 2-4 minutes per plate. Our results show that both Cell Migration Assays utilizing HT-1080 and MDA-MB-231 cell lines yield robust Z' factors suitable for high throughput screening.

Introduction, Materials and Methods

Oris™ Cell Migration Assays utilize cell seeding stoppers to create a 2 mm circular Detection Zone in the center of each well of a 96-well plate into which cell movement can occur. The Oris™ assay platform provides tissue culture treated and various Extracellular Matrix (ECM) coatings for cell migration including type I collagen and fibronectin. We studied HT-1080 and MDA-MB-231 cells migrating on the three different surfaces provided in an Oris™ Cell Migration Assay TriCoated plate in response to the application of different levels of the MEK inhibitor U0126 and in an Oris™ Pro Cell Migration Assay Collagen I coated plate in response to the actin polymerization inhibitor Cytochalasin D.

- 1: Seed HT-1080 and MDA-MB-231 cells (35,000 or 30,000 cells/well respectively) in the Oris™ Cell Migration Assay plates (see Figure 1 for plate layout) and incubate at 37°C/5% CO₂ to allow for cell attachment.
- 2: TriCoated plate - after 6 hours, remove the Oris™ Cell Seeding Stoppers from all migration-designated wells on the plate except for those designated as premigration controls, where the stoppers remain in place until the end of the experiment (Columns 1, 5, 9). Oris™ Pro plate - after 1 hour, replace media with media + Cytochalasin D or 0.1% DMSO for control. By this time, the Biocompatible Gel has dissolved.
- 3: TriCoated plate - treat migration wells with 0.1% DMSO vehicle or MEK Inhibitor U0126 at 37°C/5% CO₂ incubator for an additional 16 hour period to allow for cell migration. Oris™ Pro plate - incubate with Cytochalasin D for 18 hours at 37°C/5% CO₂ to disrupt actin formation, which inhibits cell migration.
- 4: Fix with 0.25% glutaraldehyde and permeabilize with 0.1% TritonX-100 for 15 minutes.
- 5: Rinse twice with PBS and stain cells with 1 µg/mL Propidium Iodide for 30 minutes at RT. A final wash step is unnecessary prior to scanning the plate due to the imagers' confined detection region.
- 6: Acquire well images on IsoCyte and ImageXpress Micro Systems. The IsoCyte DL System was set up using the 488 nm excitation laser and 600 nm long pass emission filter to acquire the PI signal. Whole well image acquisition was done at 5 x 5 micron resolution. ImageXpress Micro System was set up using a 2X objective and the standard Cy3 filter cube for excitation and emission. With a 2X objective, one third of the well area from a 96 well plate is imaged and the entire 2 mm diameter region-of-interest from the Oris™ plate is comfortably captured.

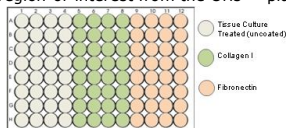


Figure 1. Oris™ Cell Migration Assay TriCoated Plate showing three different migration substrates. Rows A:D were seeded with HT-1080 cells and Rows E:H were seeded with MDA-MB-231 cells.

Results

Well images acquired from both the IsoCyte and ImageXpress Micro platforms were analyzed with a pre-optimized image process that identifies the fluorescent objects (cells) against a thresholded background within a specified region-of-interest (ROI). The area of the ROI that is occupied by cells can be graphed to show the amount of migration that occurred into the previously "stoppered" area. The IsoCyte instrument is capable of on-the-fly analysis so the final data is available as soon as the scan is completed. In this report, data is shown from a scan+analyze that was completed in 5 minutes. The ImageXpress Micro System completes the scan in 5 minutes and data is automatically saved to a secure MDCStore™ Database where it can then be processed using MetaXpress® Software with results analyzed in <7 minutes.

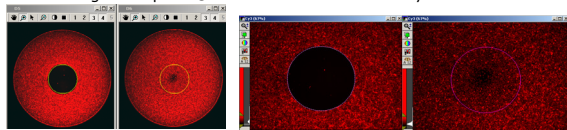


Figure 2. Representative collagen coated well images from IsoCyte DL (whole wells on left) and from ImageXpress® Micro Systems (2X magnification on right). The circular region-of-interest (ROI) is shown.

This assay showed that both HT-1080 and MDA-MB-231 cells migrated more on collagen than on the tissue culture or fibronectin coated surfaces of the Oris™ TriCoated cell migration assay plate. It can also be noted that the HT-1080 cells migrated to cover more of the ROI than did the MDA-MB-231 cells.

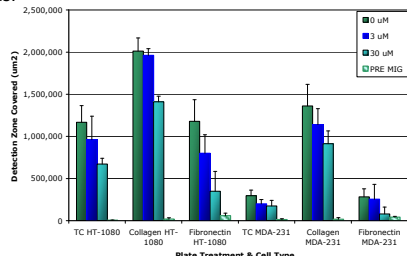


Figure 3. Graph of data from IsoCyte DL System showing that both cell types migrated more on collagen than on the tissue culture.

Results

The MEK inhibitor U0126 showed a dose dependent inhibition of cell migration on all surfaces of the Oris™ Cell Migration Assay TriCoated plate, however, the inhibition was more complete on tissue culture and fibronectin treated surfaces.

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[U0126]	HT-1080		MDA-MB-231	
	3 µM	30 µM	3 µM	30 µM
TC	24%	45%	30%	38%
Collagen	6%	33%	13%	26%
Fibronectin	31%	60%	21%	51%

Figure 4. Table shows % inhibition of cell migration compared to non-treated wells. U0126 inhibited cell migration less in collagen treated wells than the other surfaces.

Results from IsoCyte DL and ImageXpress Micro imagers were compared by reading the same Oris™ TriCoated plate on both instruments and analyzing the images with the instrument's specific analysis software. As expected, higher doses of inhibitor resulted in lower area of the region-of-interest (ROI) being occupied by fluorescent cells in both instruments and for all surface treatments.

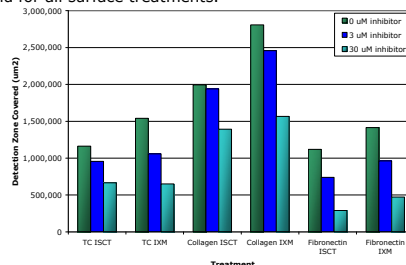


Figure 5. Graph of data from HT-1080 cells showing cell migration in response to doses of MEK inhibitor. The area covered in the Pre-migration control wells (stopper removed after treatment) was subtracted out to normalize the results between instruments. A slightly larger ROI was analyzed in the ImageXpress Micro (IXM) images than the IsoCyte (ISCT) images, accounting for the larger area covered in all cases since more of the peripheral cells were included. The Z' factor for these cells is between 0.70 and 0.85 for all treatments.

A second assay, using the Oris™ Pro Cell Migration Collagen I coated plate, was imaged and analyzed on the IsoCyte Scanning Cytometer. Since the gel dissolves and does not require removal, the Oris™ Pro plate is ideal for automation and high throughput screening. Although the entire well was imaged, the Detection Zone alone was interrogated for amount of cell migration in the presence of 1/2 log dilutions of Cytochalasin D.

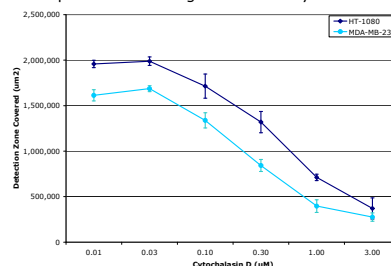


Figure 6. Inhibition of HT-1080 and MDA-MB-231 cell migration in Oris™ Pro Cell Migration Assay in response to doses of Cytochalasin D. The IC50 for HT-1080 cells was 0.34 µM and for MDA-MB-231 was 0.24 µM.

Summary

- HT-1080 and MDA-MB-231 cells demonstrate inhibition of migration in response to the MEK inhibitor U0126 and to the actin polymerization inhibitor Cytochalasin D.
- Oris™ Cell Migration Assay plate setup and sample manipulation is simple and easy to perform and can be used on live or fixed cells (live cell data not shown here).
- Oris™ Cell Migration Assay TriCoated plates provide a unique solution to investigators who want to determine optimal extracellular matrices (ECMs) for migration of specific cell lines.
- The Oris™ Pro Cell Migration Assay, with a dissolvable biocompatible gel, is well-suited for using in a screening environment.
- Both the high-throughput IsoCyte DL or ImageXpress Micro imagers have proven to be ideal for use in quantifying cell migration into a central cell-exclusion zone as shown by comparable analysis results.
- Images of the entire well can be analyzed during acquisition using the IsoCyte system.
- Images of the cell-exclusion zone acquired by the ImageXpress Micro instrument can be converted to data and stored in a secure database for easy access or further analysis.
- Higher confidence in data is possible with analysis of images compared to total intensity reads from microplate readers since cell growth can be visualized using an imaging system.
- Both imaging systems may be integrated with robotic automation for walk-away functionality.

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