

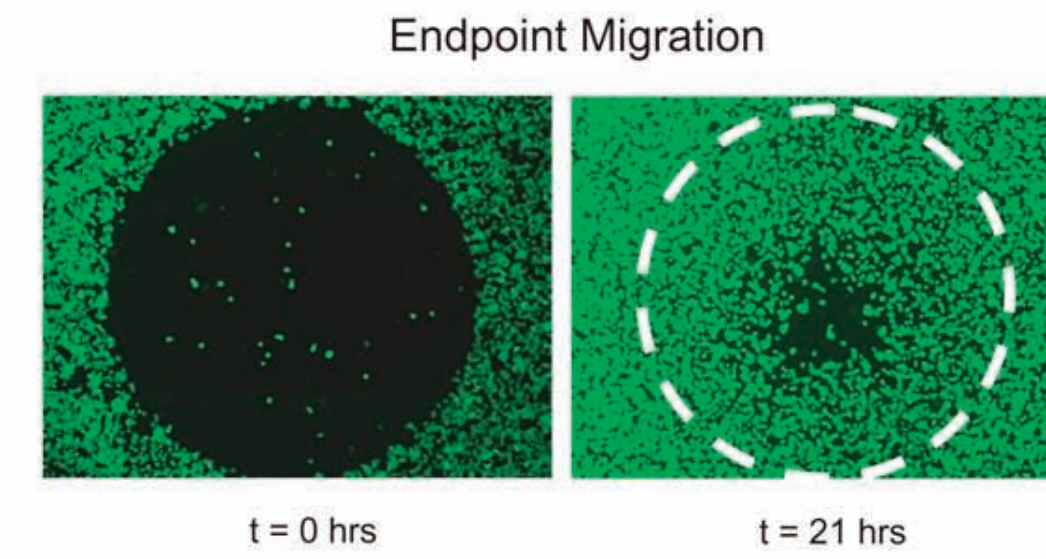
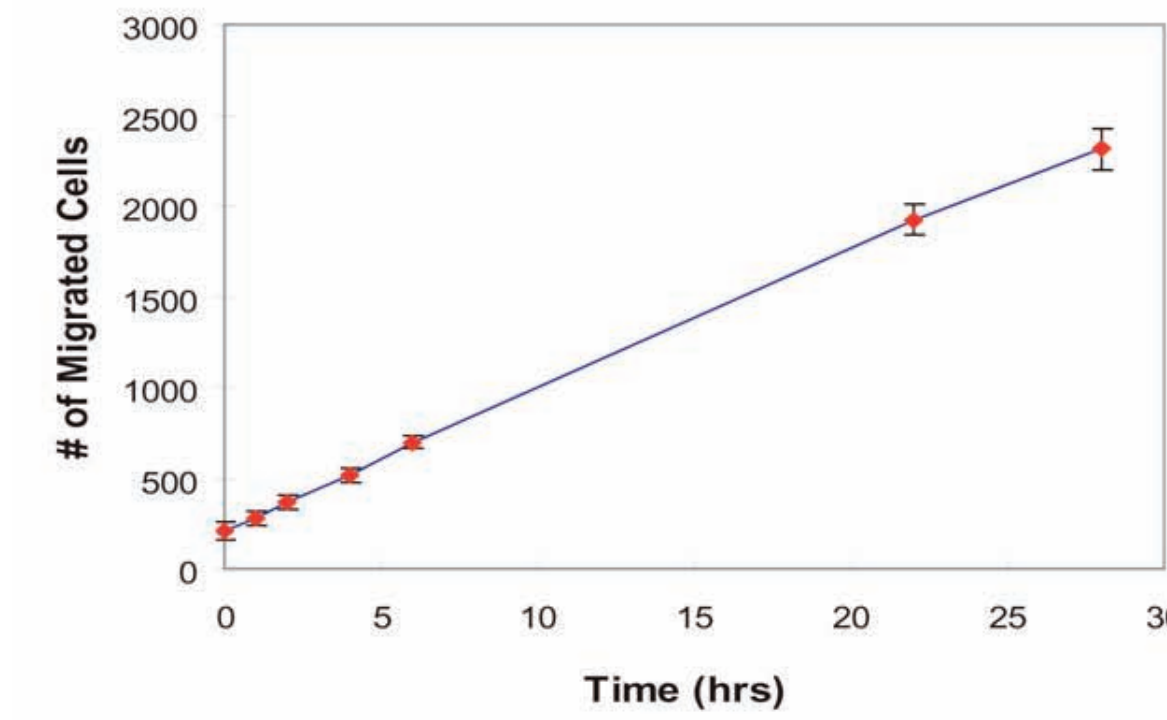
Optimizing Robustness of the Membrane-free, Oris™ Cell Migration Assay for High Throughput Screening using the BioTek Synergy™ HT Multi-Mode Microplate Reader

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Kinetics of HT-1080 Cell Migration

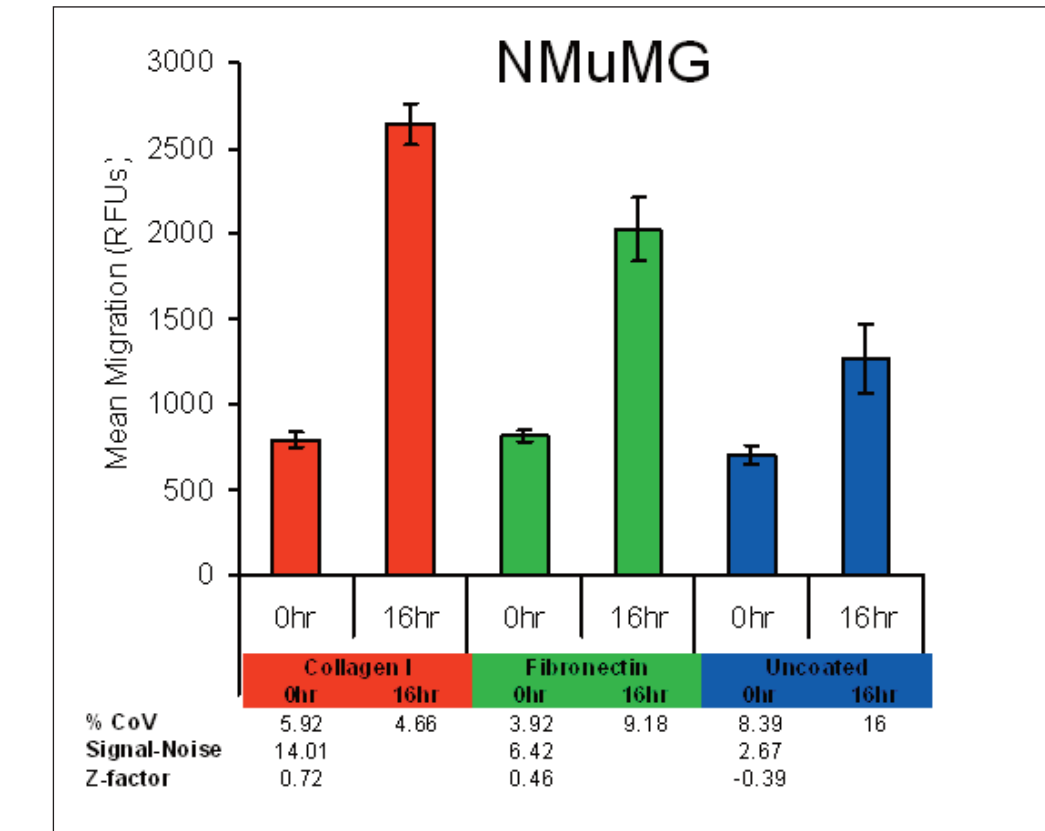


Methods: HT-1080 cells were seeded at 50,000 cells/well and allowed to adhere before stoppers were removed. The graph on the left demonstrates the kinetics of the cell migration assay at various time points. The numbers of migrated cells were interpolated from standard curves relating Cell Tracker Green (Invitrogen) fluorescence to cell number. Each time point represents the mean of nine replicate measurements. The images on the right are fluorescent photomicrographs taken at t=0 and t=21 hrs in the absence of the mask. The analytic zone into which cells have migrated is encircled by the white dotted line.

Results:

- HT-1080 cell migration is detectable in < 4 hrs.
- Migration of HT-1080 cells under these conditions is linear for up to 28 hrs, where monolayer coverage of the analytic zone is complete.

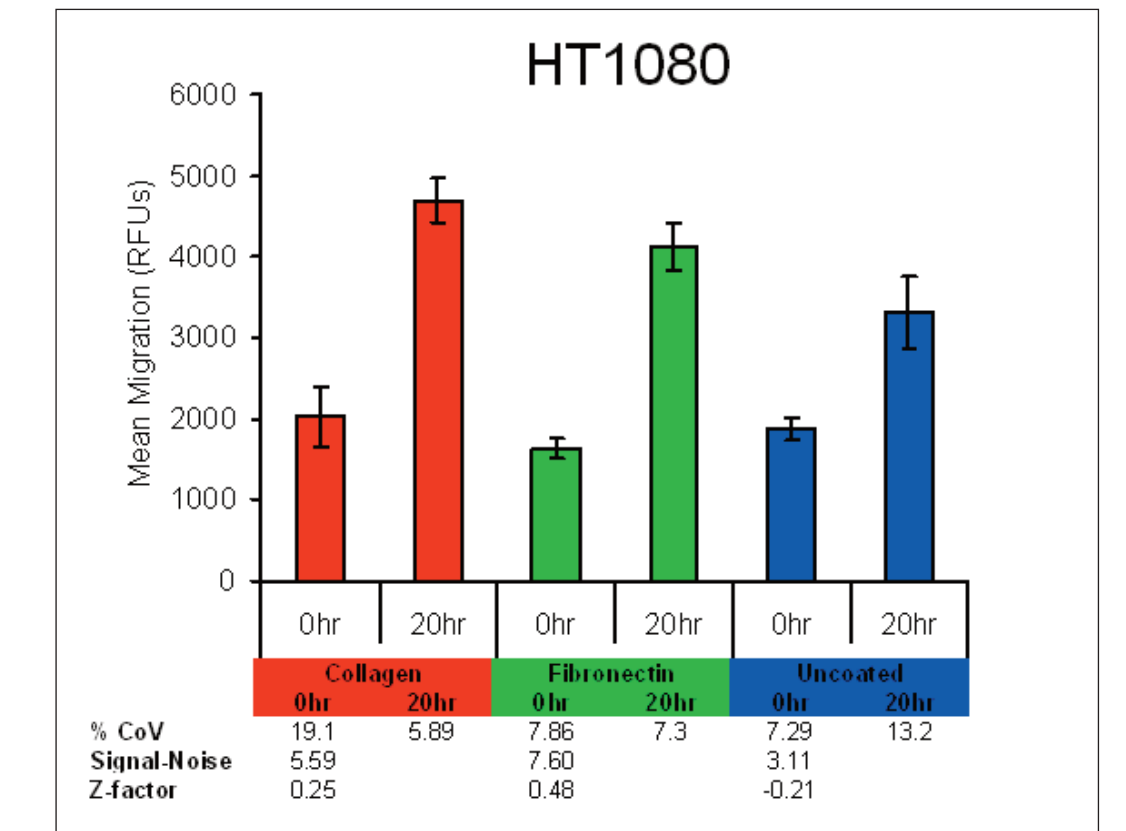
Cell Migration is Differentially Affected by ECM



Methods: NMuMG breast epithelial cells were seeded at 25,000 cells/well and allowed to attach for 6 hours on TriCoated plates. Following 16 hour migration, cells were labeled with Calcein AM (Invitrogen), and migration in the detection zone was then quantified as previously described.

Results:

- Collagen I promoted most migration for NMuMG cells.
- Fibronectin was most suitable for HT1080 cells.
- Uncoated plates supported least migration for both cell lines.
- Oris™ TriCoated Assay kits are recommended for initial Assay Optimization of migration conditions.



Methods: HT1080 human fibrosarcoma cells were seeded at 35,000 cells/well and allowed to attach for 4 hours on TriCoated plates. Following 20 hour migration, cells were labeled with Calcein AM (Invitrogen), and migration in the detection zone was then quantified as previously described.

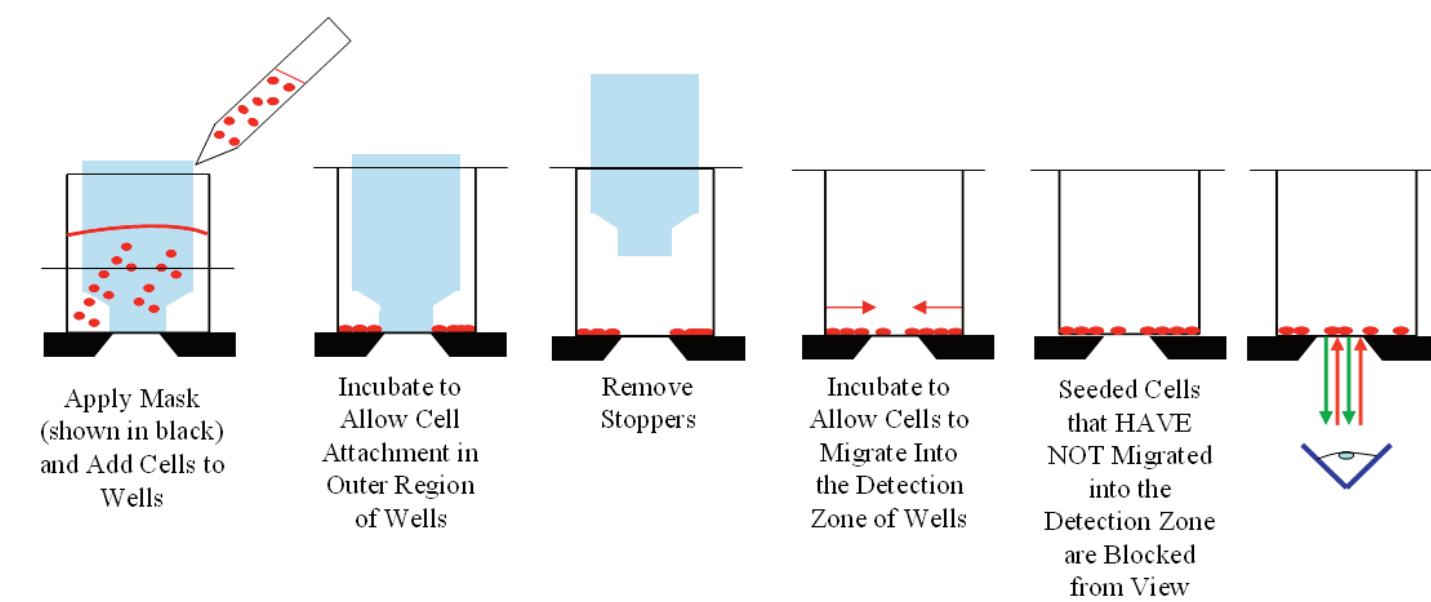
Oris™ Cell-based Assays from Platypus Technologies



Synergy™ HT from BioTek Instruments

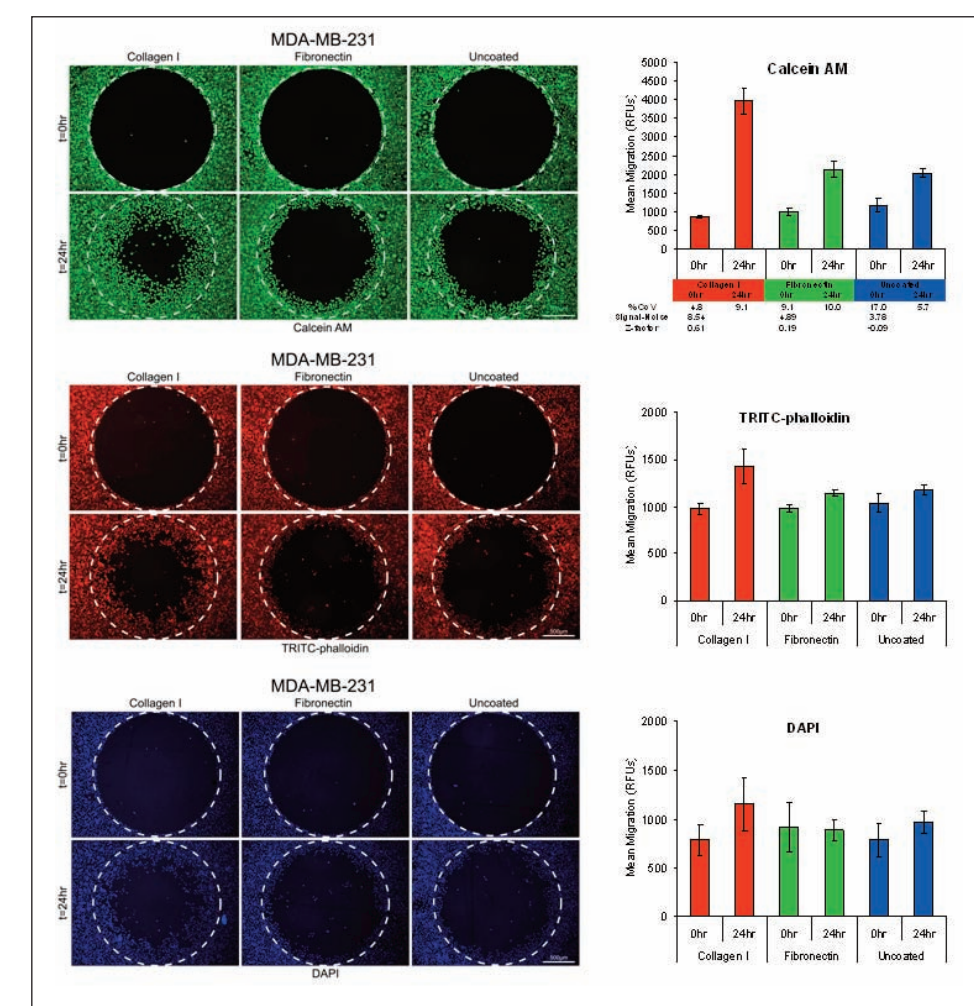


Schematic of Oris™ Cell Migration Assay



- 96-well plate populated with cell seeding stoppers (blue) that exclude cells from the central migration zone of the well.
- Opaque mask (black) restricts detection of signal to the central migration zone.
- After cell adhesion, silicone stoppers are removed and cells are permitted to migrate into the central migration zone.
- Cells are stained and detected by using a microplate reader or inverted microscope

MDA-MB-231 Cells Undergo Robust Migration on Collagen I



Methods: The Oris™ Cell Migration Assay TriCoated (Platypus Technologies, LLC) was used to assess cell migration of MDA-MB-231 breast epithelial cells. Cells were seeded at 20,000 cells/well and allowed to attach overnight onto plates coated with either type I collagen (Collagen I), fibronectin, or tissue culture treated (uncoated) wells. Once the cells formed a confluent monolayer, the silicone stoppers were removed and migration proceeded for 24 hours. Following migration, cells were labeled with Calcein AM (Invitrogen), and migration in the detection zone was then quantified by using a BioTek Synergy™ HT Multi-mode microplate reader with the Oris™ Detection Mask attached to the bottom of the plate. Next, cells were fixed and stained for filamentous actin with TRITC-phalloidin (Sigma), and a nuclear DAPI stain (Thermo Fisher). Data represent mean ± STD from a minimum of 8 wells for each condition. Images were acquired, in the absence of the mask, by use of a Zeiss Axiovert inverted microscope.

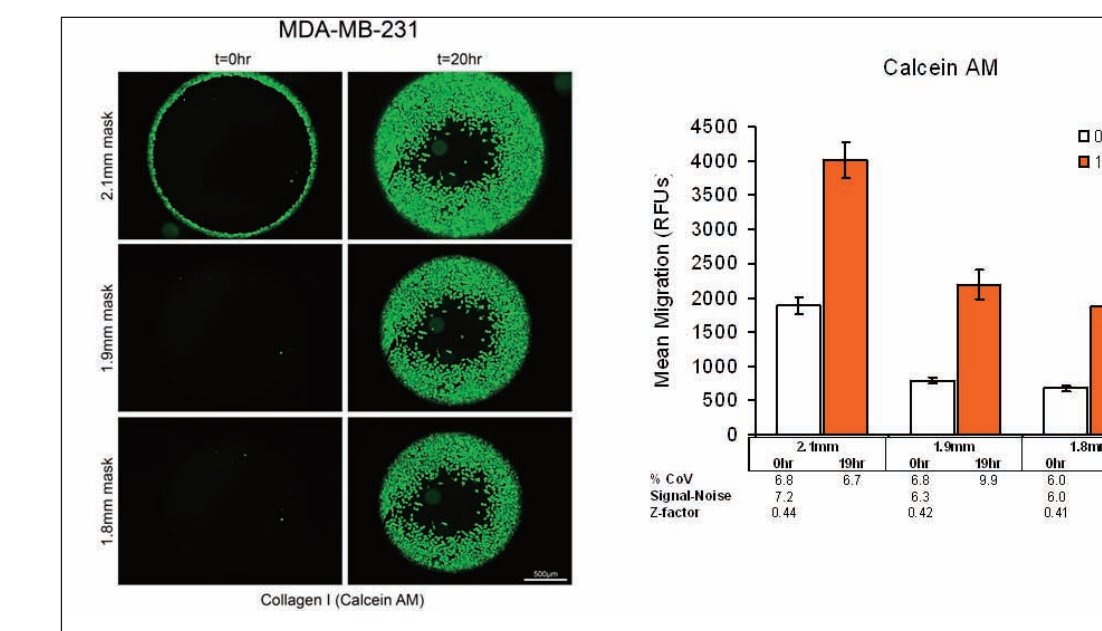
$$S:N = \frac{\text{mean of positive} - \text{mean of negative}}{\sqrt{(\text{SD of positive})^2 + (\text{SD of negative})^2}}$$

$$Z = 1 - \frac{3SD \text{ of sample} + 3SD \text{ of control}}{\text{mean of sample} - \text{mean of control}}$$

Results:

- MDA-MB-231 cells migrated more rapidly and produced a better signal-noise and z' factor on collagen I compared to fibronectin or uncoated wells.
- Using Calcein AM to quantify migration provided best results using BioTek Synergy HT Microplate Reader for MDA-MB-231 and also NMuMG and HT1080 cells.
- Synergy HT results correlated with visual images obtained by microscopy for Calcein AM staining.

Different Mask Aperture Sizes Produce Similar Trends in Signal-Noise



Methods: MDA-MB-231 breast epithelial cells were seeded at 25,000 cells/well and allowed to attach for 7 hours onto collagen I-coated wells. Once the cells formed a confluent monolayer, the silicone stoppers were removed and migration proceeded for 20 hours. Following migration, cells were labeled with Calcein AM (Invitrogen), and migration in the detection zone was then quantified as previously described.

Results:

- 2.1mm mask aperture allows the capture of early migration events and provides the optimal CoV, signal-noise, and z' factor.

Conclusions:

- Oris™ Cell Migration Assay allows for sample throughput and assay performance suitable for HTS.
 - 96-well plate design
 - z' factors suitable for screening
- Oris™ TriCoated plates are recommended for assay optimization.
 - Cell migration is cell line specific and is differentially affected by plate coatings
- BioTek Synergy HT is a recommended microplate reader for use with the Oris™ Cell Migration Assay.
 - CellTracker™ Green allows for kinetic readings of cell migration
 - Calcein AM is an optimal stain for end point readings of cell migration
 - The 2.1mm mask aperture yields optimal CoV, signal-noise and z'