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

Keren I. Hulkower[1], Renee L. Herber[1], Scott Gehler[1], Paul Held, Ph.D[2] and Xavier Amouretti[2]

1. Platypus Technologies, Madison, WI, 2. BioTek Instruments, Inc., Winooski, VT

BioTek

Abstract

Dysregulated cellular migration has been implicated in the failure of diabetic wound healing and in metastasis of cancer cells. Identification of potential therapeutic compounds that regulate cell motility would benefit from improved methods for high throughput screening (HTS).

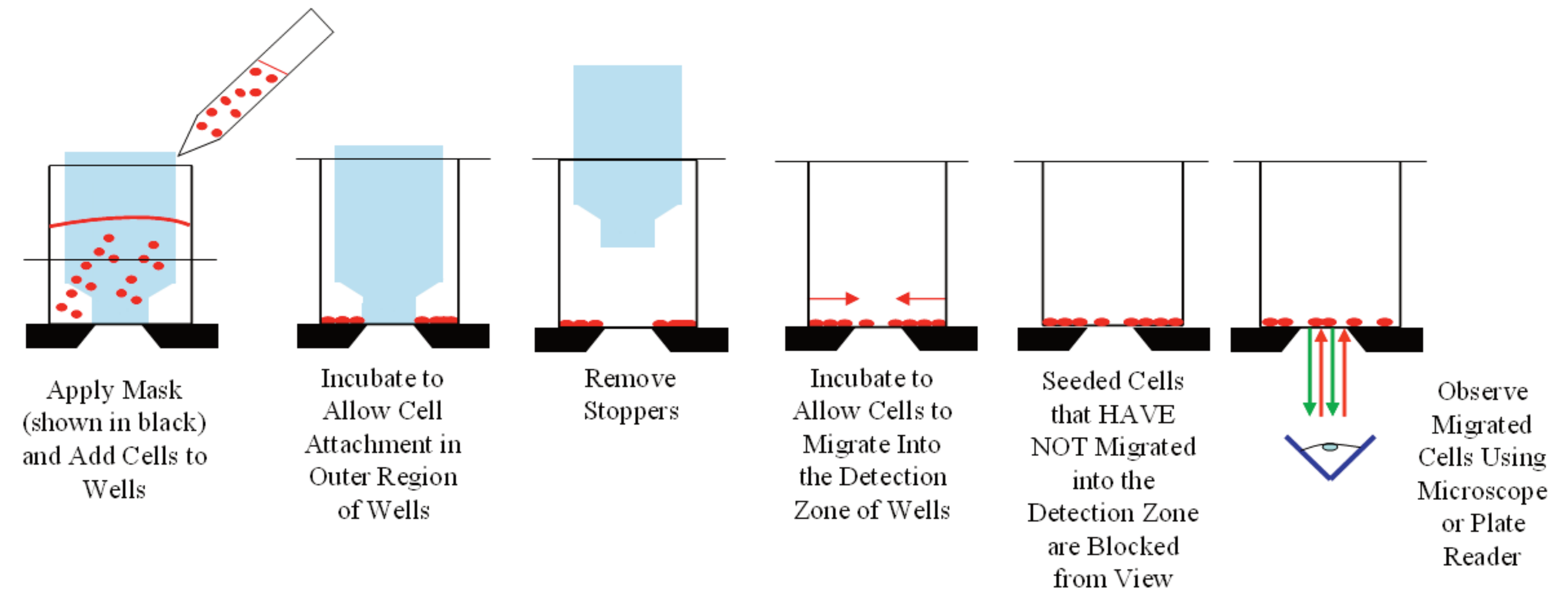
The Oris™ Cell Migration Assay comprises a 96-well plate with silicone stoppers in each well that facilitate seeding cells annularly while excluding them from a 2 mm diameter centrally located detection zone. Following cell seeding and cell attachment, the stoppers are removed and cells migrate into the detection zone. Cells are then stained and an opaque mask, providing apertures that align with the detection zones, is attached to the bottom of the plate. The fluorescent signal is measured by using BioTek Instruments Synergy™ HT Multi-Mode Microplate Reader. Capture of fluorescence is limited to cells that have migrated into the detection zone based upon restrictive apertures of the opaque mask.

We demonstrate here the differential effects of (i) cell seeding on fibronectin, collagen I and tissue culture treated polystyrene substrates; (ii) staining with cytoplasmic, nuclear and cytoskeletal fluorescent dyes; and (iii) using a variety of mask aperture sizes on the robustness of the Oris™ Cell Migration Assay with HT-1080, MDA-MB-231 and NMuMG cells. Z-factors of > 0.5 were achieved for some combinations of these test parameters demonstrating the value of the Oris™ Cell Migration Assay for HTS applications.

Oris™ Cell-based Assays from Platypus Technologies

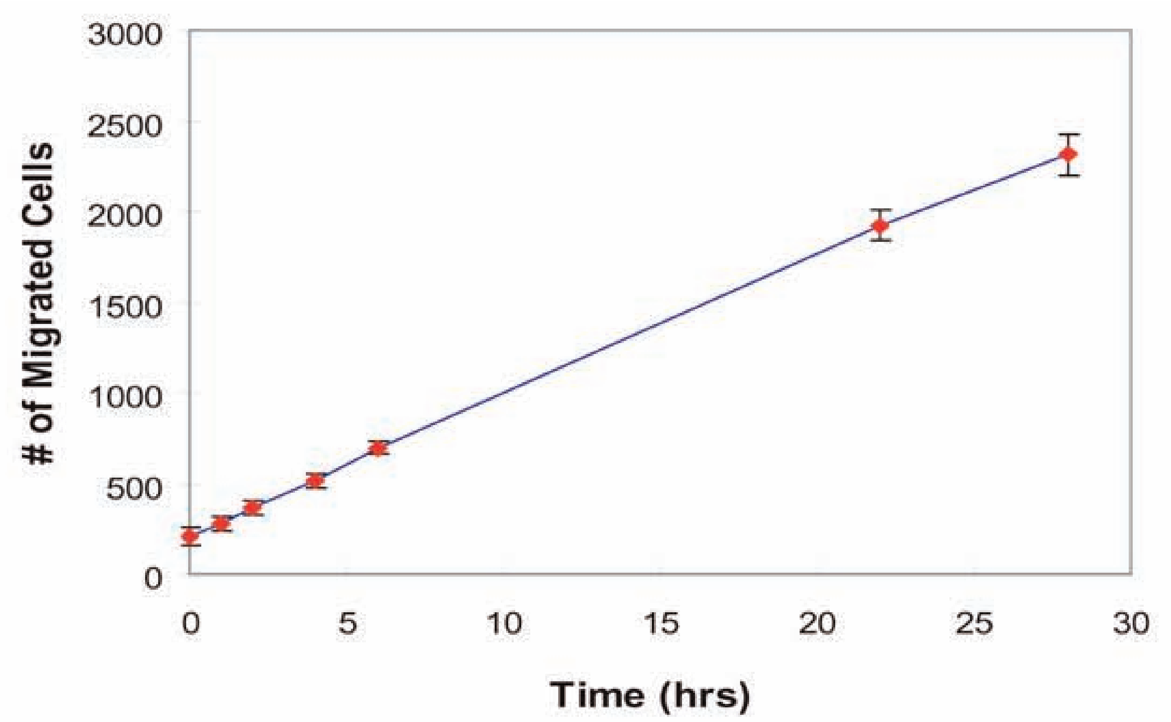
Synergy™ HT from BioTek Instruments

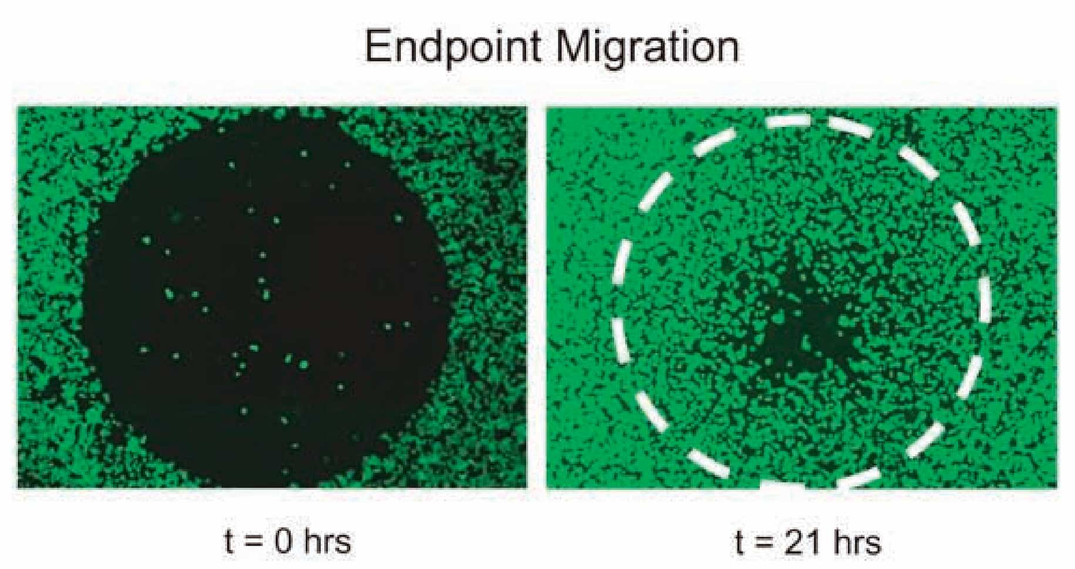
Schematic of Oris™ Cell Migration Assay


Apply Mask (shown in black) and Add Cells to Wells
Incubate to Allow Cell Attachment in Outer Region of Wells
Remove Stoppers
Incubate to Allow Cells to Migrate Into the Detection Zone of Wells
Seeded Cells that HAVE NOT Migrated into the Detection Zone are Blocked from View
Observe Migrated Cells Using Microscope or Plate Reader

- 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

Kinetics of HT-1080 Cell Migration



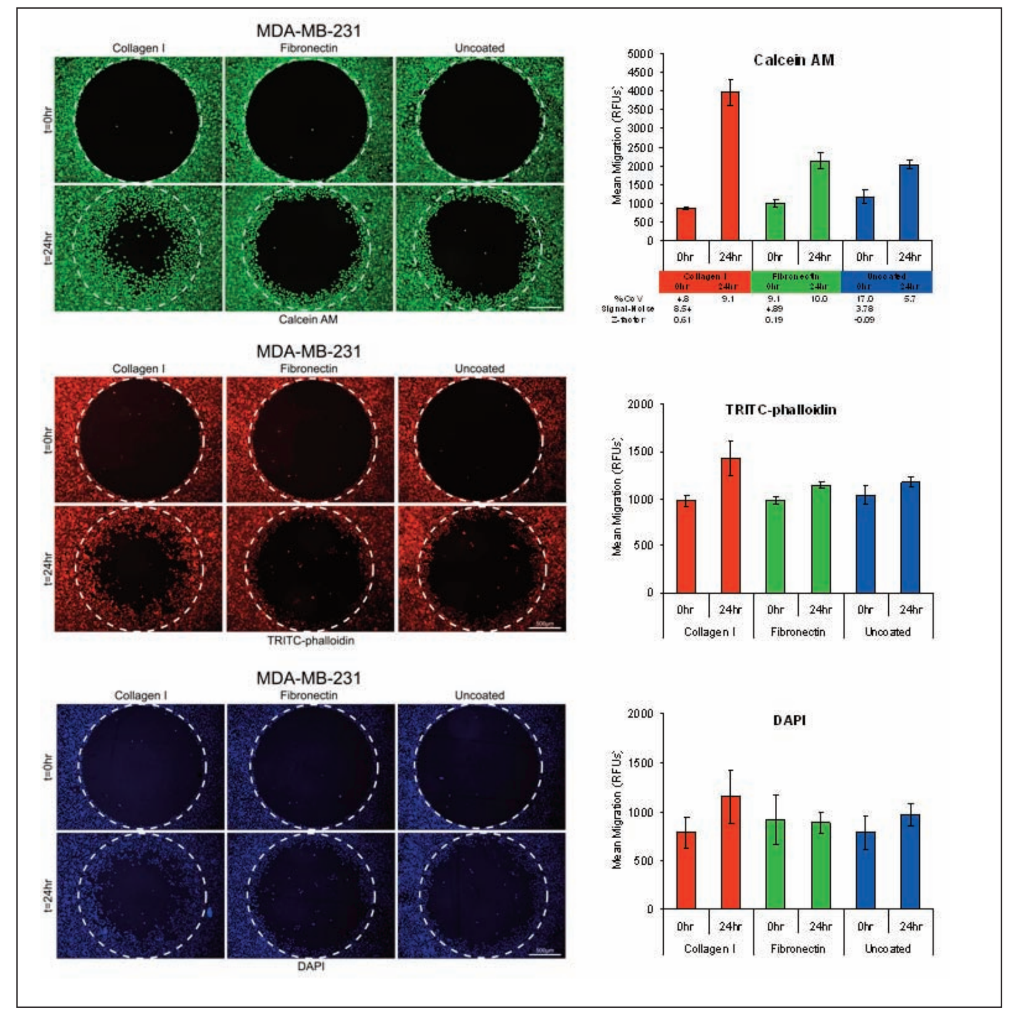


Methods: HT-1080 cells were seeded at 50,000 cells/well and allowed to adhere before stoppers were removed. The graph on the left demonstrates the kinetics of the cell migration assay at various time points. The numbers of migrated cells were interpolated from standard curves relating Cell Tracker Green (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.

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



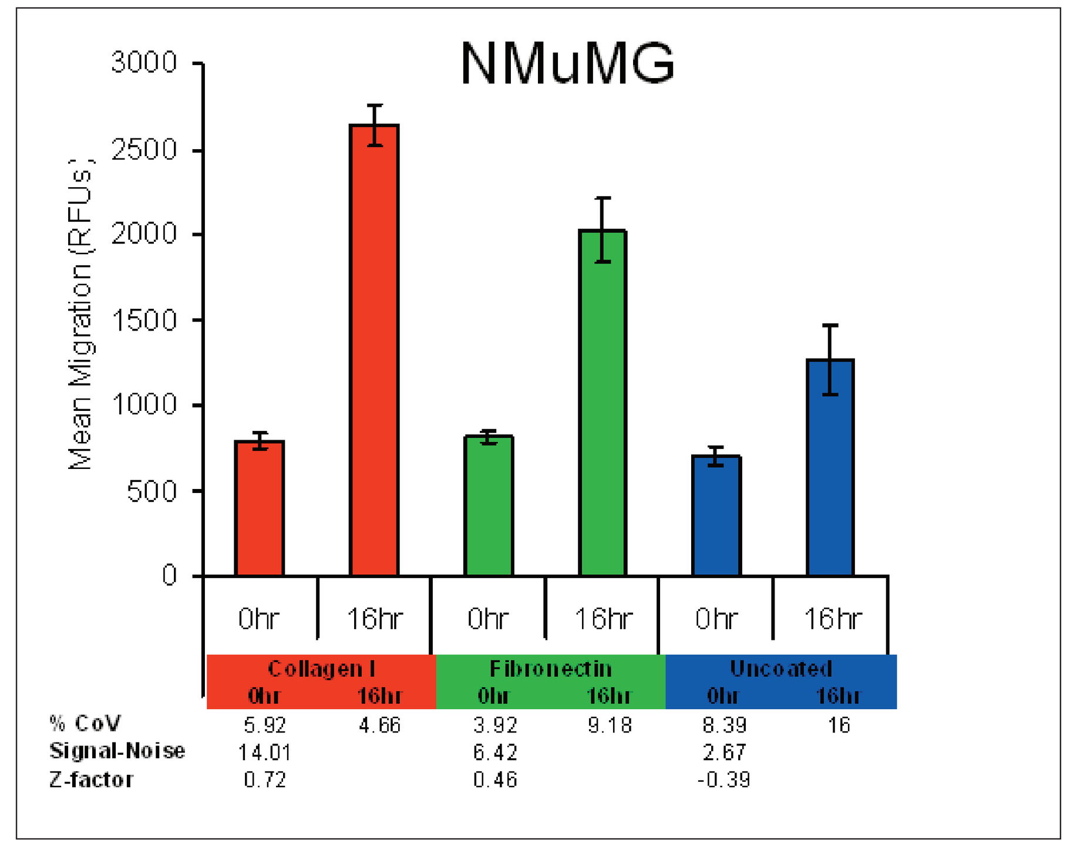
Methods: The Oris™ Cell Migration Assay TriCoated (Platypus Technologies, LLC) was used to assess cell migration of MDA-MB-231 breast epithelial cells. Cells were seeded at 20,000 cells/well and allowed to attach overnight onto plates coated with either type I collagen (Collagen I), fibronectin, or tissue 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.

$$S{:}N = \frac{\text{mean of positive} - \text{mean of negative}}{\sqrt{(\text{SD of positive})^2 + (\text{SD of negative})^2}} \qquad Z = 1 - \frac{3\text{SD of sample} + 3\text{SD of control}}{\text{mean of sample} - \text{mean of control}}$$

Results:

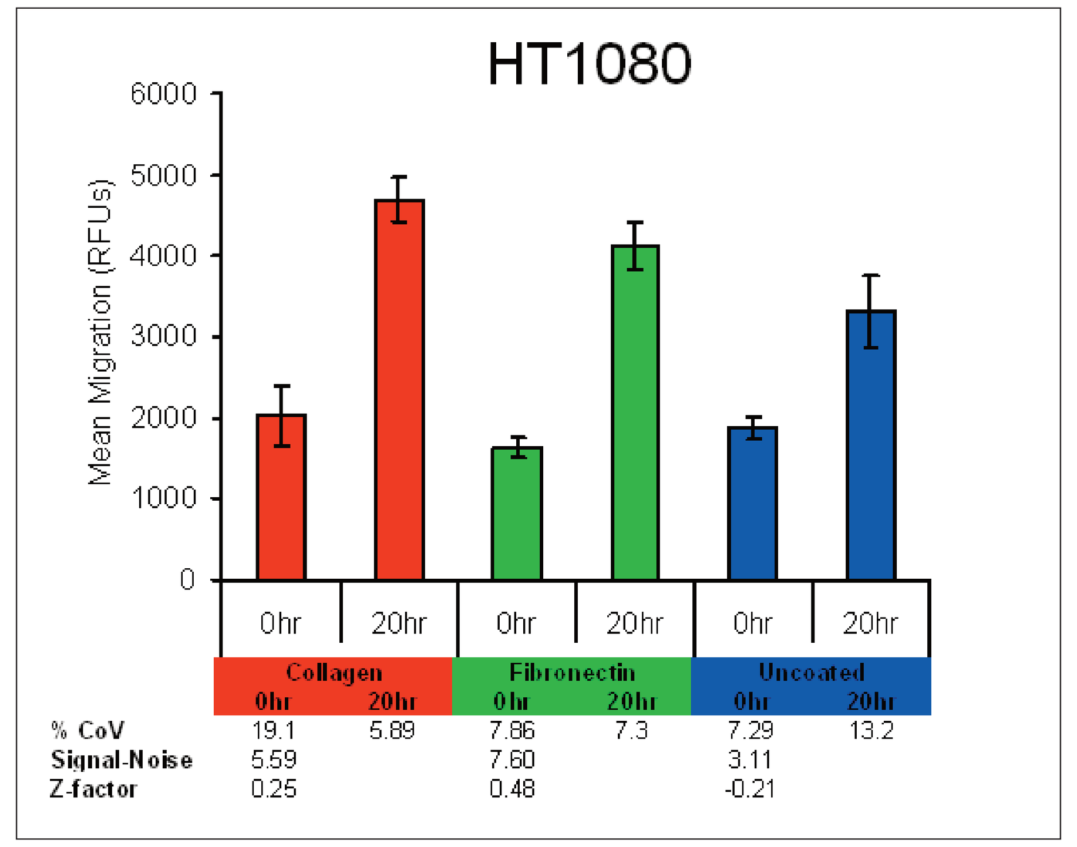
- MDA-MB-231 cells migrated more rapidly and produced a better signal-noise and z' factor on collagen I compared to fibronectin or uncoated wells.
- Using Calcein AM to quantify migration provided best results using BioTek Synergy HT Microplate Reader for MDA-MB-231 and also NMuMG and HT1080 cells.
-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

Cell Migration is Differentially Affected by ECM



	Collagen I 0hr	Collagen I 16hr	Fibronectin 0hr	Fibronectin 16hr	Uncoated 0hr	Uncoated 16hr
% CoV	5.92	4.66	3.92	9.18	8.39	16
Signal-Noise	14.01		6.42		2.67	
Z-factor	0.72		0.46		-0.39	

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.



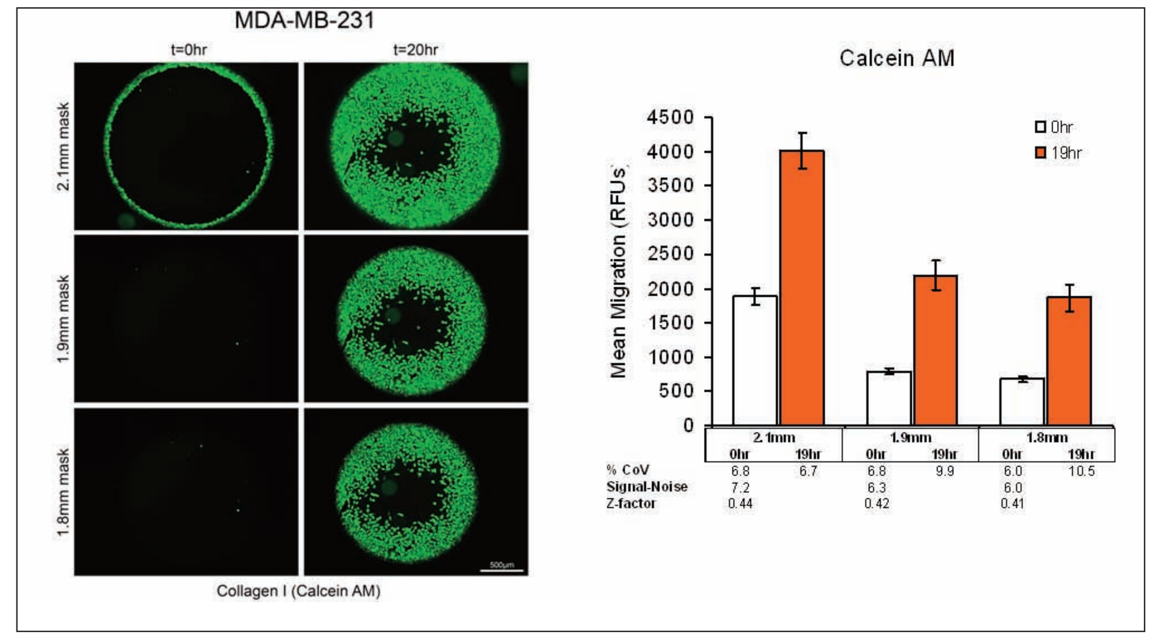
	Collagen 0hr	Collagen 20hr	Fibronectin 0hr	Fibronectin 20hr	Uncoated 0hr	Uncoated 20hr
% CoV	19.1	5.89	7.86	7.3	7.29	13.2
Signal-Noise	5.59		7.60		3.11	
Z-factor	0.25		0.48		-0.21	

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.

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.

Different Mask Aperture Sizes Produce Similar Trends in Signal-Noise



	2.1mm 0hr	2.1mm 19hr	1.9mm 0hr	1.9mm 19hr	1.8mm 0hr	1.8mm 19hr
% CoV	6.8	6.7	6.8	9.9	6.0	10.5
Signal-Noise	7.2		6.3		6.0	
Z-factor	0.44		0.42		0.41	

Methods: MDA-MB-231 breast epithelial cells were seeded at 25,000 cells/well and allowed to attach for 7 hours on 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'