BioTek
Get a Better Reaction.

Application Note

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

Keren I. Hulkower, Renee L. Herber, and Scott Gehler, Platypus Technologies, Madison, WI
Paul Held, Ph.D and Xavier Amouretti, BioTek Instruments, Inc., Winooski, VT

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 is a microplate-based tool suitable for screening. The 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.

Introduction

Cell migration is a fundamental activity intrinsic to development and maintenance of homeostasis in processes such as wound healing, neovascularization and the workings of the immune system. The failure of cells to migrate, or the movement of invasive cells into inappropriate locations, is central to many disease processes such as dysregulated wound healing and cancer. Additionally, understanding cell migration is critical to emerging technologies such as tissue engineering and the successful bio-integration of prosthetic devices. There is a need for new technologies that will enable migration and invasion assays that are more reliable, less labor intensive, consume fewer cells and reagents, can be performed in less time and are amenable to high throughput formats.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.

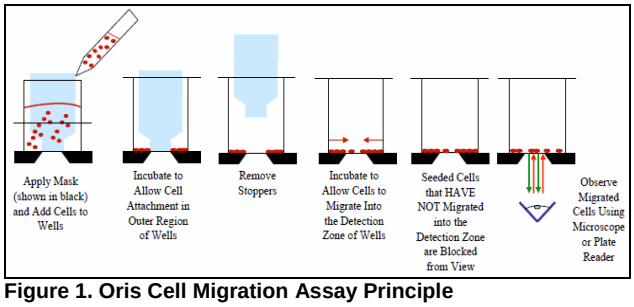


Figure 1. Oris Cell Migration Assay Principle

The Oris™ Cell Migration Assay (Figure 1) is a multistep process that starts with the application of a mask to the bottom of the wells of a 96-well microplate. In addition, a polymeric insert is fitted to the inside of the wells of the microplate. This insert prevents cells from seeding the inner analytic zone of the well when cells are added to the wells of the microplate. After cell attachment has occurred, the inserts are removed, allowing cells to freely migrate into the central analytic zone of the wells.
Cells that have not migrated into the analytic zone are blocked from view by the mask initially applied to the plate bottom. Migrated cells are analyzed by microscopy or by detection with a Synergy HT Multi-Mode Microplate Reader using fluorescence mode.

Methods

Kinetics of HT-1080 Migration

HT-1080 cells were pre-treated with mytomycin C (MMC) for 2 hours to inhibit proliferation and then stained with 2.5 mM Cell Tracker Green (Invitrogen). The cells were seeded at 50,000 cells/well in media containing 10% Fetal Bovine Serum (FBS) and allowed to adhere. Inserts were removed, and the plate was incubated to permit cell migration. The numbers of migrated cells were interpolated from standard curves relating Cell Tracker Green fluorescence to cell number and measured on Synergy HT. Each time point represents the mean of nine replicate measurements.

MDA-MB-231 cell migration optimization using Oris TriCoated Plates

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.

NMuMG and HT1080 cell migration optimization using Oris TriCoated Plates

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 and migration in the detection zone was then quantified as previously described. 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 and migration in the detection zone was then quantified as previously described.

Oris Mask Aperture Size Effect

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.

For all experiments, S/N ratios and z' factors were calculated according to the following equations:

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

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

Results

Kinetics of HT-1080 Migration

Initial experiments demonstrated the kinetics of cell migration into the center analytical zone.

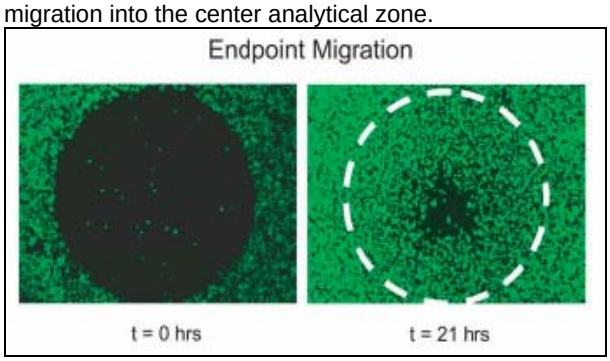


Figure 2. Fluorescent photomicrographs taken at t=0 and t=21 hrs in the absence of the mask. The analytic zone into which cells have migrated is encircled by the white dotted line.

As demonstrated in Figure 2, FBS-stimulated, non-proliferating HT1080 cells almost completely fill the analytical zone within 21 hours of removal of the mask. Furthermore, when the migration is examined over time, migration was detectable in less than 4 hours and linear over a 28.5-hour period (Figure 3).

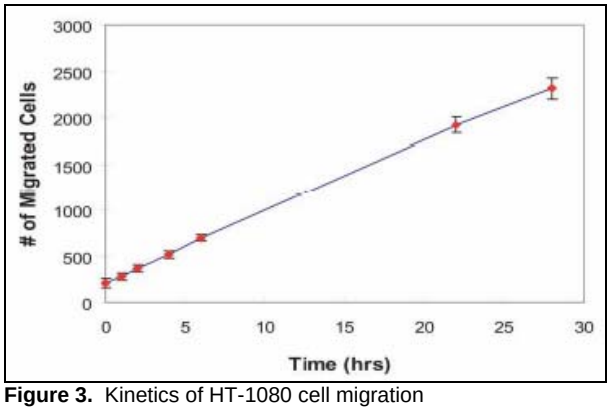


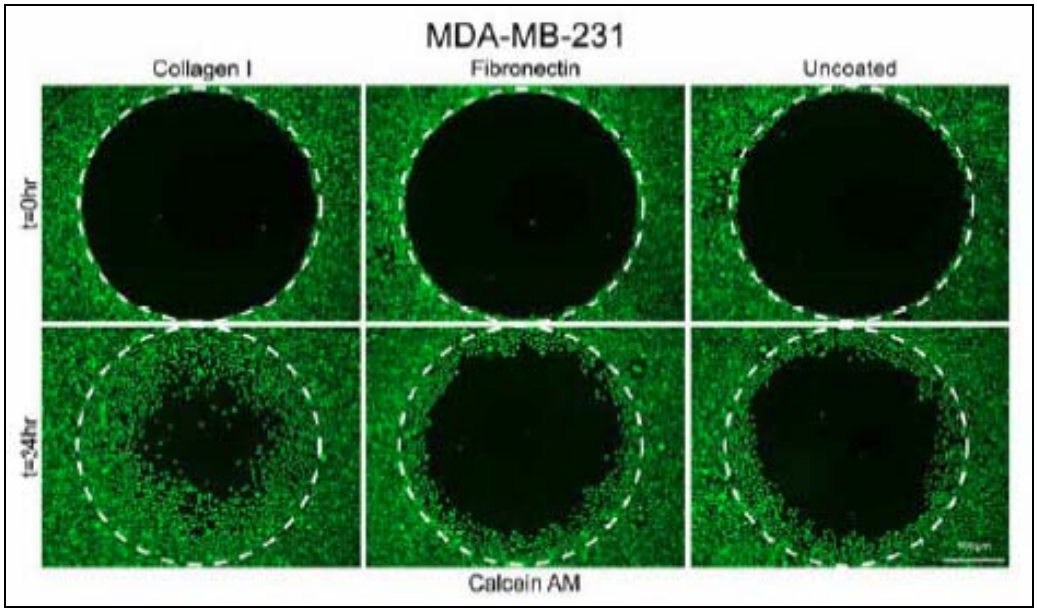
Figure 3. Kinetics of HT-1080 cell migration

MDA-MB-231 cell migration optimization using Oris TriCoated Plates

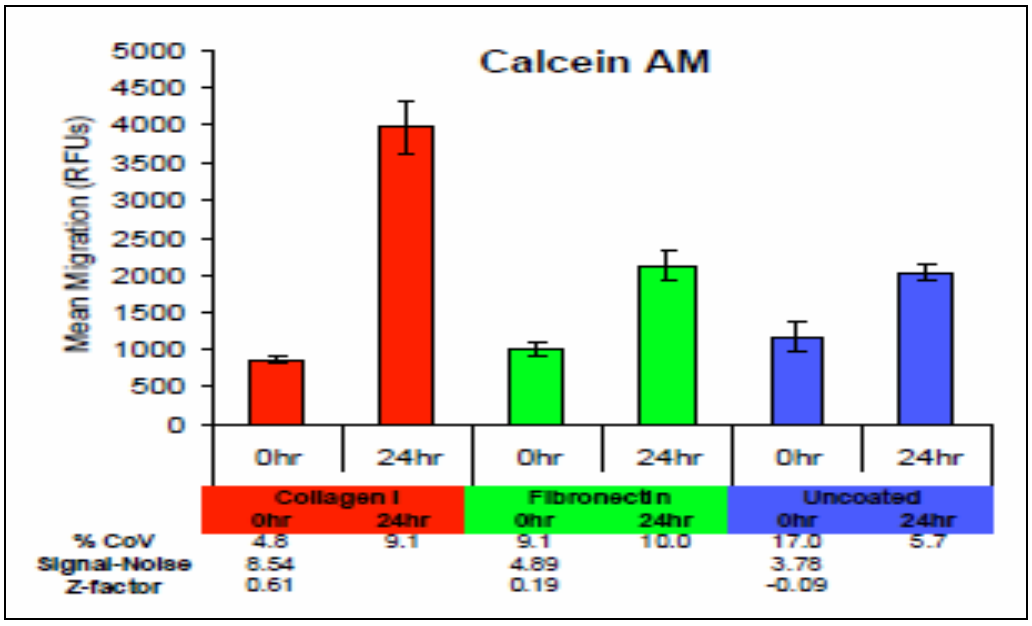
MDA-MB-231 cell migration is demonstrated in Figure 4 both visually and as a fluorometric readout from Synergy HT. It is apparent from the inverted microscope images for all three cellular stains that the most cell migration is occurring with the collagen I coating of the TriCoated plates. For the Synergy HT readings, there is a definite preference for using the Calcein AM cellular stain. All three stains provide similar background readings (cell migration at t = 0), but the Calcein AM stain provides greater intensities for cells which migrate into the detection volume defined by the mask aperture. It is evident however, that collagen I is the best surface for enhanced cell migration in agreement with the images. CVs in replicate measurements were less than 10% and S/N and z' factors were consistent with screening applications.

Figure 4.

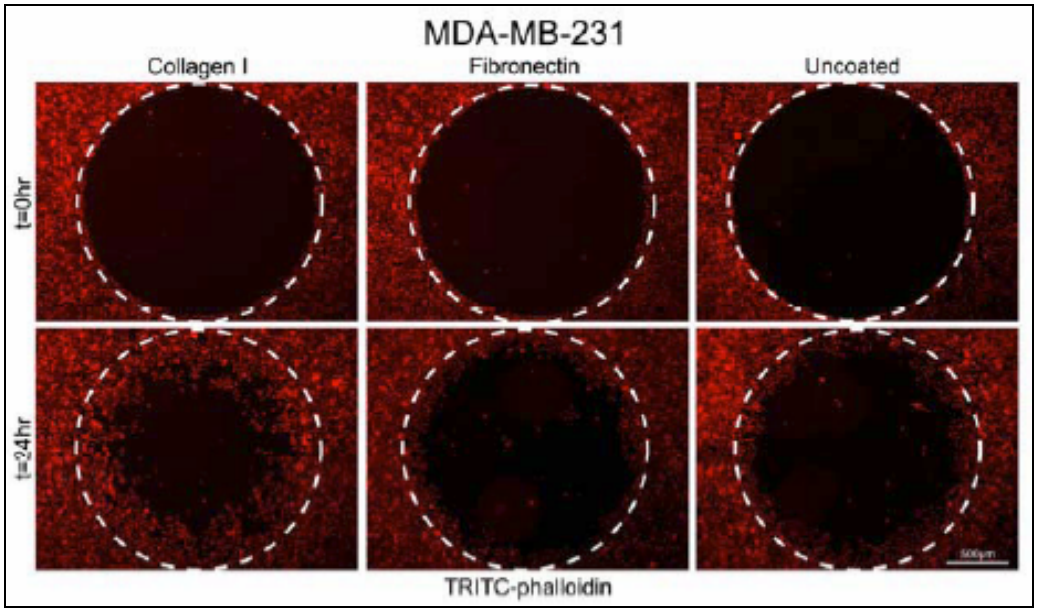
A


MDA-MB-231
Collagen I
Fibronectin
Uncoated
t=0hr
t=24hr
Calcein AM

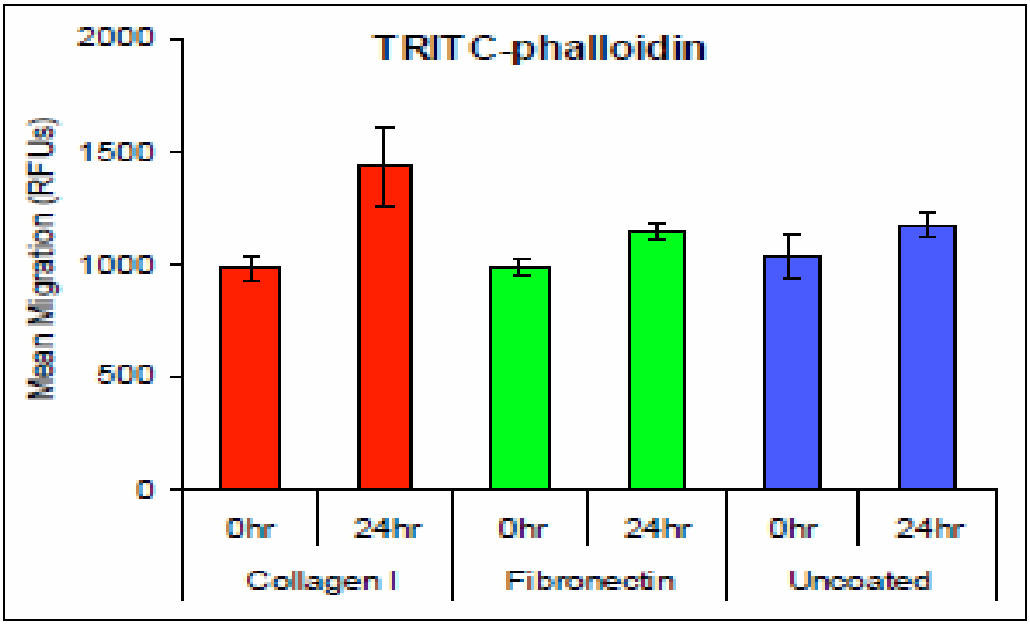
B


Calcein AM
Mean Migration (RFUs)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0hr
24hr
0hr
24hr
0hr
24hr
Collagen I
Fibronectin
Uncoated
0hr 24hr 0hr 24hr 0hr 24hr
% CoV 4.8 9.1 9.1 10.0 17.0 5.7
Signal-Noise 8.54 4.89 3.78
Z-factor 0.61 0.19 -0.09

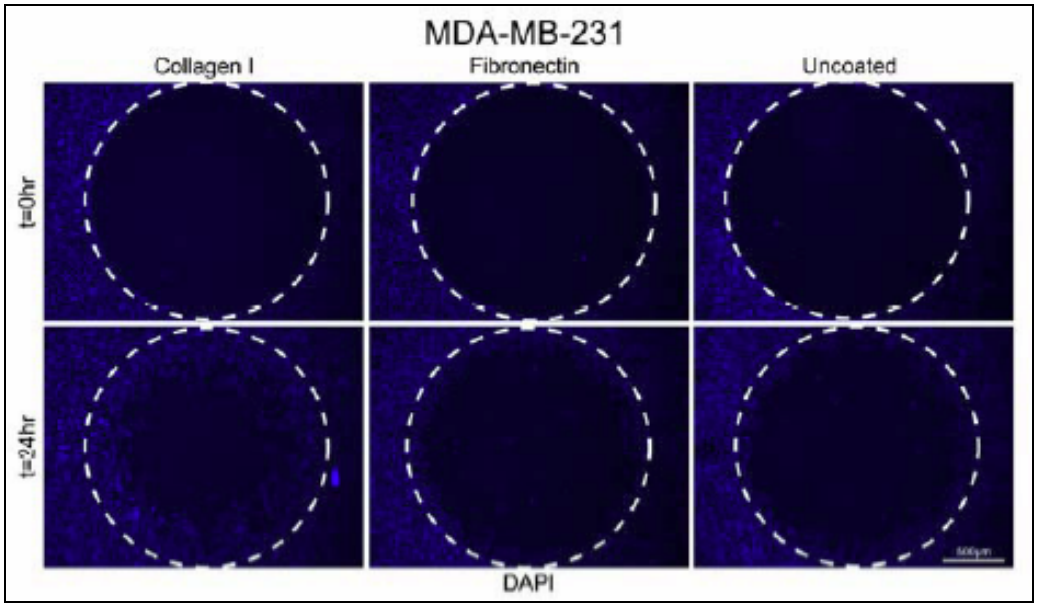
C


MDA-MB-231
Collagen I
Fibronectin
Uncoated
t=0hr
t=24hr
TRITC-phalloidin

D


TRITC-phalloidin
Mean Migration (RFUs)
2000
1500
1000
500
0
0hr
24hr
0hr
24hr
0hr
24hr
Collagen I
Fibronectin
Uncoated

E



F

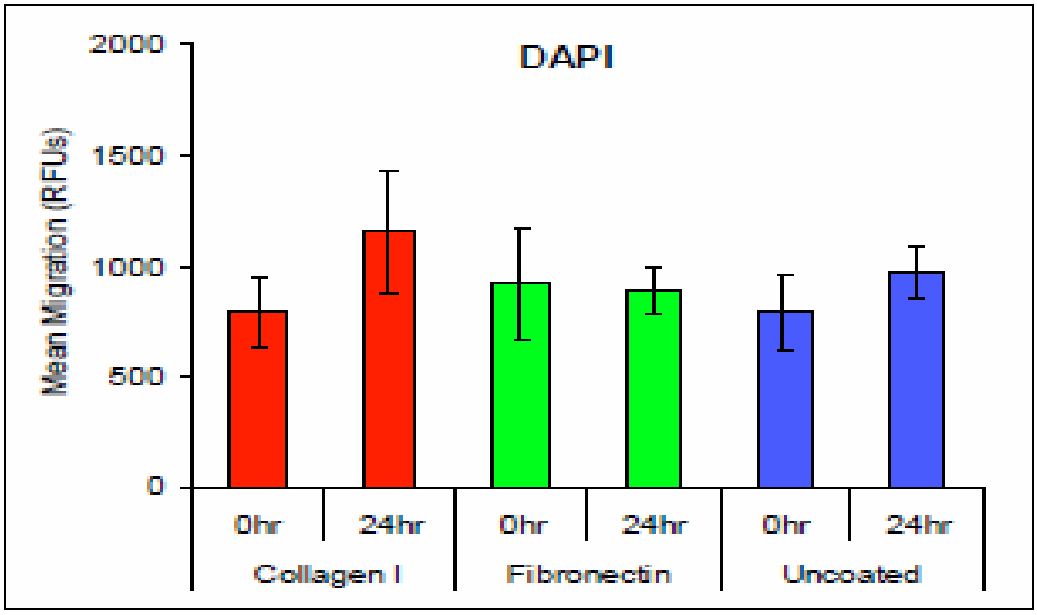
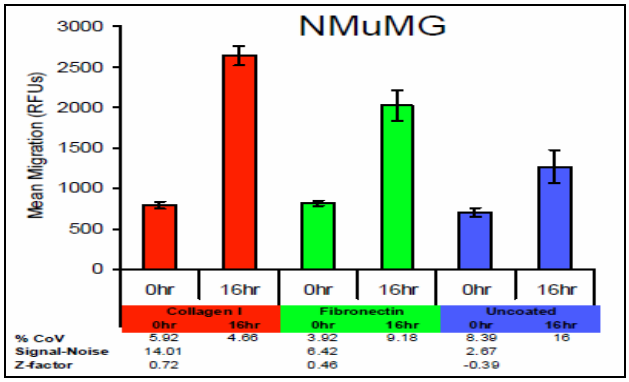


Figure 4. MDA-MB-231 breast epithelial cell migration as measured by inverted microscope (A, C, E) and Synergy HT Multi-Mode Microplate Reader (B, D, F). Three different cellular stains were used: Calcein AM (A, B), TRITC-phalloidin (C, D), and a nuclear DAPI stain (E, F). In A-F, the three different surfaces of the TriCoated plates were tested (Collagen I, Fibronectin and uncoated).

NMuMG and HT1080 cell migration optimization using Oris TriCoated Plates

Different cell types tend to have different cell migration behavior, which is why the TriCoated plates were developed to allow for the optimization of surface for specific cell type. In this study, NMuMG and HT1080 cell migration behavior was monitored using the Synergy HT and TriCoated plates. It is evident from Figure 5 that different surfaces are optimal for the two cell lines: NMuMG cells provide better performance with collagen I; while fibronectin appears to be a better surface for HT1080 cells. It is recommended that for new assays using untested cell lines one should start with TriCoated plates to optimize surface.



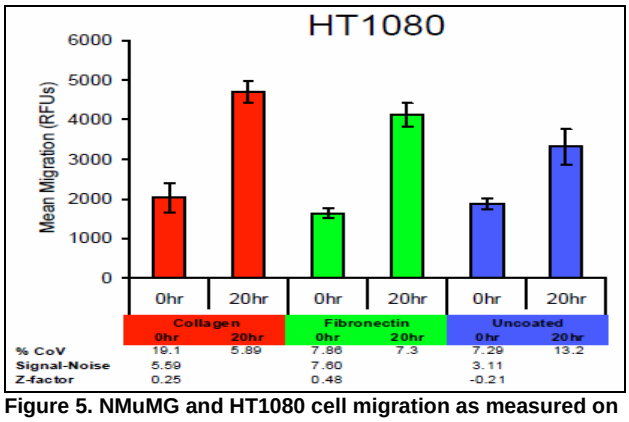
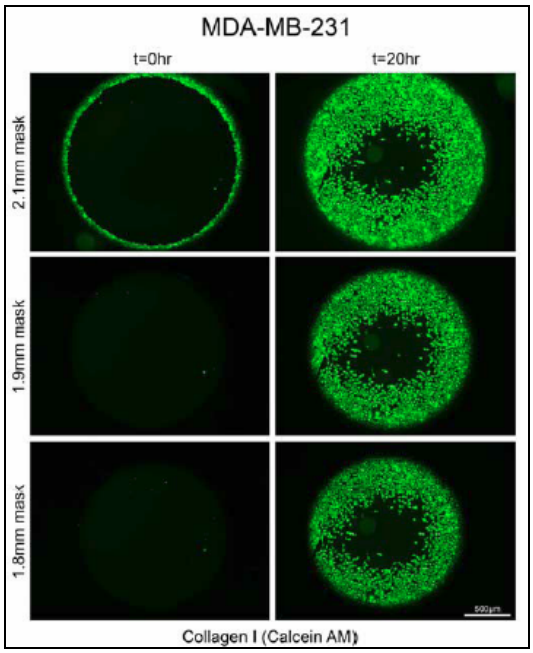


Figure 5. NMuMG and HT1080 cell migration as measured on Synergy HT.

Oris Mask Aperture Size Effect

Figure 6 demonstrates the effect of different mask aperture sizes on cell migration signals obtained by both microscopy and Synergy HT. It is evident that although the background is higher for the larger aperture mask, it does provide the best performance. This mask aperture size would also allow for the quantification of early cell migration events also.

A



B

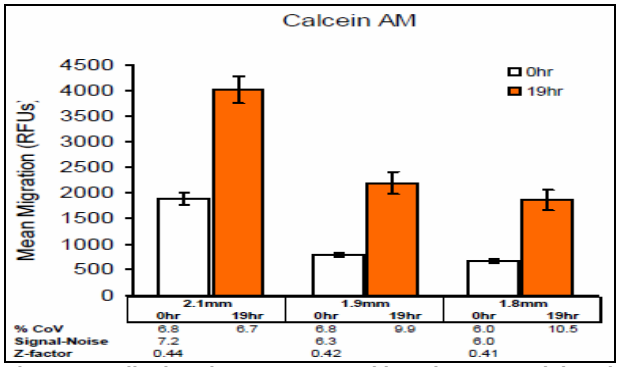


Figure 6. Cell migration as measured by microscopy (A) and Synergy HT (B) for different mask aperture sizes.

Discussion

The Oris Cell Migration Assay based on a 96-well microplate design allows for sample throughput and assay performance suitable for HTS when used in conjunction with microplate readers, such as the Synergy HT Multi-Mode Reader. Cell migration is cell line specific and thus it is highly recommended to test cell migration on the different surfaces provided in the TriCoated microplates. Calcein AM is the dye recommended for use with Synergy HT.