Effect of Mask Aperture Size on Robustness of Oris™ Cell Migration Assay Data

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Technical Memo

Summary

An Oris™ Detection Mask is provided with Oris™ stopper- based assay kits to enable data capture using a fluorescence microplate reader. The Oris™ Detection Mask has apertures with a 2.1 mm diameter while the diameter of the Detection Zone created by the stoppers is 2.0 mm. While the larger aperture allows for detection of early cell migration events, it also allows a background fluorescence signal from seeded cells that have not migrated. When viewed under a microscope with the Oris™ Detection Mask in place, pre-migration reference wells are observed to have either an annular corona or crescent-like fluorescence pattern depending upon the offset between the mask apertures and Detection Zones. Using a series of masks with 1.8, 1.9 and 2.1 mm aperture sizes, we demonstrate that the crescent-like fluorescence patterns observed when using the Oris™ Detection Mask in place do not affect the robustness of the cell migration data obtained using a fluorescence microplate reader. While 1.9 and 1.8 mm mask apertures reduce the background fluorescence units in pre-migration reference wells, the fluorescence signal from migrating cells is likewise reduced by the smaller apertures. The resulting trends include: higher coefficients of variance in migration wells with lower signal-to-noise ratios and Z′-factors for the assays as the aperture size is decreased. Thus, the crescent-like fluorescence patterns that may be observed with the Oris™ Detection Mask provided in Oris™ stopper-based assay kits do not negatively impact quantitative results.

Introduction

In addition to microscopes and high content imagers, Oris™ stopper-based kits can be used with a microplate reader to quickly quantify cell migration. In order to do so, an Oris™ Detection Mask must be attached to the bottom of the assay plate and a microplate reader equipped with a bottom probe must be used. The apertures of the Oris™ Detection Mask restrict visualization of the cells in the Detection Zone of each well. The Oris™ Detection Mask provided in Oris™ stopper-based assay kits has an aperture size that is 0.1 mm larger in diameter than the 2.0 mm sized Detection Zone created by the stoppers. This background level of fluorescence from stained cells, provided by the slightly oversized apertures, is intended to ensure that fluorescence detection falls within the linear range of the microplate reader so as to detect the earliest movements into the Detection Zone by migrating cells.

One of the consequences of slightly oversized apertures is that users will observe background fluorescence in pre-migration reference wells when the assay plate is viewed under a microscope with the Oris™ Detection Mask attached. This background fluorescence is visible as either an annulus or a crescent-like shape depending upon the alignment of the apertures with the Detection Zones. While all Oris™ stopper based products must meet established release specifications for centering of the silicone tips within the assay wells, crescents can be expected to be observed. This technical memo serves to demonstrate the validity and robustness of the microplate reader data obtained from assay plates using the 2.1 mm aperture masks in comparison to prototype masks having 1.8 and 1.9 mm apertures.

Methods

Masks: A standard Oris™ Detection Mask with 2.1 mm apertures was used in this study along with similar masks providing 1.8 and 1.9 mm apertures.

Cell Migration: MDA-MB-231 breast epithelial cells (25,000 cells/ 100 µL well) were seeded onto Collagen I coated plates in the Oris™ Cell Migration Assay (Figure 1).

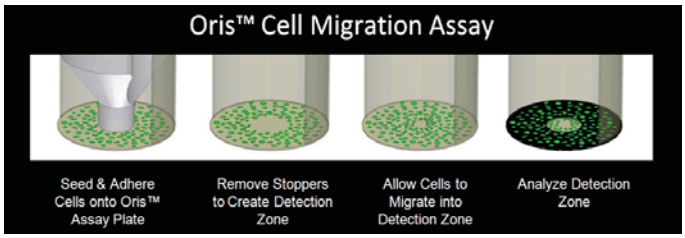

Figure 1: Oris™ Cell Migration Assay Schematic

After 7 hours, the Oris™ Cell Seeding Stoppers were removed from the test wells and the cells incubated for 20 hours to allow migration to proceed. Similarly, HT-1080 fibrosarcoma cells (32,500 cells/100 µL well) were seeded onto Tissue Culture treated plates in the Oris™ Cell Migration Assay and allowed to attach for 4 hours prior to Oris™ Cell Seeding Stopper removal. Migration of the HT-1080 cells was allowed to proceed for 19 hours. At the end of the migration period, the cells were stained with Calcein AM for 30 minutes. For pre-migration references, Oris™ Cell Seeding Stoppers remained in a portion of wells in the Oris™ plates until staining. Both assay plates were subjected to a successive series of readings with each of the 3 masks on a BioTek Synergy™ HT microplate reader. Data were analyzed to obtain coefficients of variance, signal-to-noise (S:N) ratios and Z′ factors (shown below). Images of assay wells were captured using a Zeiss Axiovert fluorescence microscope equipped with a CCD camera.

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

Results

We conducted experiments with two different cell lines, MDA-MB-231 and HT-1080, on collagen I coated and tissue culture treated Oris™ Cell Migration Assay plates, respectively. In each study, half of the wells served as pre-migration references where the Oris™ Cell Seeding Stoppers remained in place until the end of the incubation period while migration was allowed to progress overnight in the other half of the wells. The cells were stained with Calcein AM, representative images acquired and microplate reader relative fluorescence unit values were captured using masks with 2.1, 1.9 and 1.8 mm apertures.

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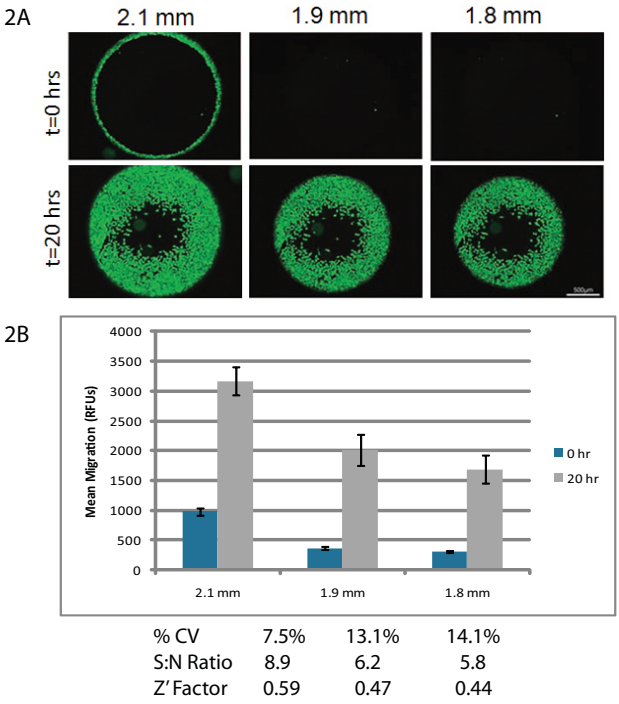
An annular ring of fluorescence was observed in a representative image of a pre-migration reference well of MDA-MB-231 cells with the 2.1 mm aperture Oris™ Detection Mask affixed to the plate bottom (Figure 2A). This fluorescent ring was not visible when the 1.9 mm and 1.8 mm aperture test masks were employed (Figure 2A). In migration wells, fluorescently labeled cells were visible using all three masks. The difference in size of the visible field was readily observable between aperture diameters (Figure 2A). Microplate reader data showed a reduction in background fluorescence in both pre-migration and full migration wells along with corresponding decreases in both the signal-to-noise ratio and Z′ factor as aperture size decreased (Figure 2B). The 2.1 mm apertures on the Oris™ Detection Mask provide the highest signal-to-noise ratio (8.9), the most robust Z′ factor (0.59) and lowest %CV (7.5%). The coefficient of variance (%CV) increased for migration wells as the aperture size decreased.

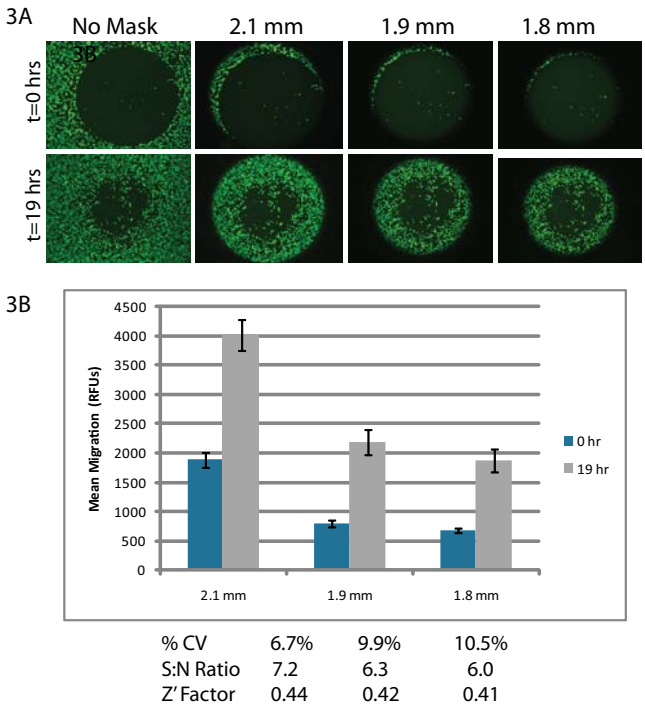


% CV	7.5%	13.1%	14.1%
S:N Ratio	8.9	6.2	5.8
Z′ Factor	0.59	0.47	0.44

Figure 2: Effect of Mask Aperture Size on Images (A) and Microplate Reader Data (B) Obtained from MDA-MB-231 Cells Migrating on Oris™ Collagen I Coated Assay Plates. Cells were labeled with Calcein AM and representative images and microplate reader data acquired using masks with 3 different aperture sizes. Microplate reader data is the average relative fluorescence units (RFUs) of 8 wells of each condition. Results indicate that decreasing the mask aperture size from 2.1 mm to 1.8 mm causes increases in the coefficient of variance (%CV) for migration wells with concomitant decreases in calculated S:N ratio and Z′ factor.

Similar findings were observed with HT-1080 cells on tissue culture treated Oris™ Cell Migration Assay plates. Figure 3A shows a representative image captured without any mask in place in which a symmetrical 2 mm Detection Zone is apparent immediately after stopper removal.

With the 2.1 mm aperture diameter Oris™ Detection Mask in place, a crescent-like pattern of fluorescence was observed in a representative pre-migration reference well. This crescent was less apparent in the pre-migration reference wells with the 1.9 mm and 1.8 mm masks attached to the assay plate, while the observed field size also diminished in the corresponding migration wells (Figure 3A). Lower relative fluorescence units were obtained by the microplate reader with decreasing aperture sizes in both pre-migration and migration assay wells (Figure 3B) with concomitant decreases in both the S:N ratio and Z′ factors and increases in the %CV values for migration wells (Figure 3B). For the HT-1080 cells, the 2.1 mm Oris™ Detection Mask provided a 7.2 S:N ratio, a Z′ factor of 0.44 and a %CV of 6.7% from the collected microplate reader data (Figure 3B).



% CV	6.7%	9.9%	10.5%
S:N Ratio	7.2	6.3	6.0
Z′ Factor	0.44	0.42	0.41

Figure 3. Effect of Mask Aperture Size on Images (A) and Microplate Reader Data (B) Obtained from HT-1080 Cells Migrating on Oris™ Tissue Culture Treated Assay Plates. Cells were labeled with Calcein AM and representative images and microplate reader data acquired using masks with 3 different aperture sizes. Microplate reader data is the average relative fluorescence units (RFUs) of 48 wells of each condition. Results indicate that decreasing the mask aperture size from 2.1 mm to 1.8 mm causes increases in the coefficient of variance (%CV) for migration wells with concomitant decreases in calculated S:N ratio and Z′ factor.

Conclusions

These experiments demonstrate that more robust data is obtained from microplate reader data captured from Oris™ Cell Migration Assays using the 2.1 mm Oris™ Detection Masks provided with the kits as compared to test masks having 1.9 and 1.8 mm apertures. Reducing the aperture size to 1.9 or 1.8 mm reduced the S:N ratio and Z′ factors and increased assay variability. While the annular or crescent-shaped background fluorescence patterns observed visually under the microscope with the 2.1 mm Oris™ Detection Mask attached may not be aesthetically appealing, this phenomenon in no way detracts from the validity and robustness of microplate reader data that can be obtained from the Oris™ Cell Migration Assays. Alternate techniques based on image analysis are also available to capture data from the Oris™ assays, which do not require the use of the Oris™ Detection Mask. These include the use of a microscope with ImageJ software or a high content imaging device with software that can define and analyze cells within the Detection Zone.