How to Measure Area Closure Using the Oris™ Pro Cell Migration Assay

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Application Note

Introduction

Cell migration is an integrated process that is critical during many physiological events, including embryonic development, tissue regeneration, and the inflammatory response. Furthermore, aberrant cell motility contributes to many pathological processes such as tumor metastasis and atherosclerosis (1).

The Oris™ Pro Cell Migration Assay (Figure 1) utilizes a 96-well plate that contains a non-toxic, dissolving biocompatible gel (BCG) that excludes cells from attaching to the centers of the well. After cells have adhered and the BCG dissolves, a cell-free zone (Detection Zone) is revealed into which cells can migrate.

While there are many assay formats to study cell migration, analysis of the results can be subjective and time consuming. **In this application note, we describe the use of ImageJ software, a freeware image analysis program developed at the National Institutes of Health (http://rsb.info.nih.gov/ij), to automatically measure the area of the Detection Zone from phase-contrast and fluorescence images and to calculate area closure in the Oris™ Pro Cell Migration Assay.**

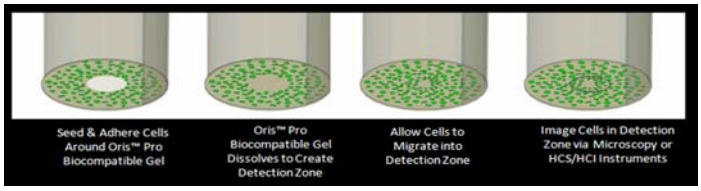

Figure 1. Oris™ Pro Cell Migration Assay Schematic

Materials & Methods

HT-1080 fibrosarcoma cells, MDA-MB-231 breast epithelial cells, and human umbilical vein endothelial cells (HUVECs) were each cultured on Oris™ Pro Collagen I and Tissue Culture (TC)-treated plates. Following cell attachment (1 hr, collagen I; 2 hr, TC), pre-migration wells were fixed with 0.25% glutaraldehyde. For drug experiments, media was removed following cell attachment and replaced with media containing vehicle only (control), 100μM UO126 (Promega), or 10μM H-1152 (Calbiochem). All migration wells were incubated for 18 hours (collagen I) and 24 hours (TC), followed by fixation. HUVECs cultured on the Oris™ Pro TC-treated plate were stained for F-actin with TRITC-phalloidin (Sigma). Phase and fluorescence images were captured using a 5X objective on a Zeiss Axiovert 200 inverted microscope with CCD camera.

Determination of Area from Phase Images*

Cell migration was assessed by measuring the area of the detection zone at the pre-migration and migration time-points using ImageJ v1.42l analysis software. The following protocol was modified as described by Kees Straatman (2). First, the image scale was set using the known micron/pixel values (***Analyze-->Set Scale***). Next, ***"Find Edges"*** (***Process-->Find Edges***) was run followed by ***"Sharpen"*** (***Process-->Sharpen***). Then, the threshold was set for each image to enhance the contrast between the Detection Zone and the cell monolayer (***Image-->Adjust-->Threshold***). In the threshold window, ***"B&W"*** was activated. ***"Apply"*** was selected in the threshold window, to convert the thresholded image to a binary image. At this point, the Detection Zone was black and the cell monolayer was white. Next, the menu command ***Process-->Find Edges*** was applied. Finally, the menu command ***Image-->Lookup Tables-->Invert LUT*** was performed.

The Wand (tracing) tool was clicked on the Detection Zone until the region was outlined (indicated by a yellow trace). Using the menu command ***Analyze-->Set Measurements***, the ***"Area"*** and ***"Display Label"*** were selected. Finally, the menu command ***Analyze-->Measure*** was performed. The resulting area measurements from the Summary Window (i.e., Area) were exported into Windows Excel for statistical analysis. The area of the Detection Zone for each condition was averaged from 24 wells. Cell migration was determined as percent closure, which was calculated as follows:

$$\left(\frac{(\text{Pre-migration})_{\text{area}} - (\text{Migration})_{\text{area}}}{(\text{Pre-migration})_{\text{area}}}\right) \times 100$$

Determination of Area from Fluorescence Images*

To measure the area of the Detection Zone from fluorescence images, the image scale was set using the known micron/pixel values (***Analyze-->Set Scale***). Next, the threshold was set to enhance the contrast between the Detection Zone and the cell monolayer (***Image-->Adjust-->Threshold***). By selecting ***"Apply"*** in the threshold window, the thresholded image was converted to a binary image. ***The Wand (tracing) tool*** was clicked on the Detection Zone until the region was outlined (indicated by a yellow trace). Using the menu command ***Analyze-->Set Measurements***, the ***"Area"*** and ***"Display Label"*** were selected. Finally, the menu command ***Analyze-->Measure*** was performed. The resulting area measurements from the Summary Window (i.e., Area) were exported into Windows Excel for statistical analysis. The area of the Detection Zone for each condition was averaged from 16 wells. Area closure was calculated as described above.

Additional information regarding the use of ImageJ for measuring area can be found at http://rsbweb.nih.gov/ij/docs/menus/analyze.html#ap.

***Pre-recorded ImageJ macros are available for both phase and fluorescence approaches at www.platypustech.com.**

Results

ImageJ analysis software was used to automatically trace and measure the area of the Detection Zone from phase-contrast and fluorescence images. In this application note, HT-1080 cells, MDA-MB-231 cells, and HUVECs were permitted to migrate on collagen I and TC surfaces in the Oris™ Pro Cell Migration Assay. Cell migration was assessed by area closure of the Detection Zone using ImageJ. Figure 2 shows example phase images of HT-1080 cells at the pre-migration (Fig 2A) and migration (Fig 2B) time points. Using ImageJ, a series of steps were applied to enhance the contrast of the phase images that were then converted to binary images (Fig 2C and 2D) and used to create area drawings via accurate tracing of the Detection Zone (Fig 2E and 2F). Using the measured areas of the Detection Zones at the pre-migration and migration time points, cell migration was quantified by calculating the percent area closure. Figure 2G shows the amount of migration of the different cells cultured on collagen I and TC-treated plates in the Oris™ Pro Cell Migration Assay.

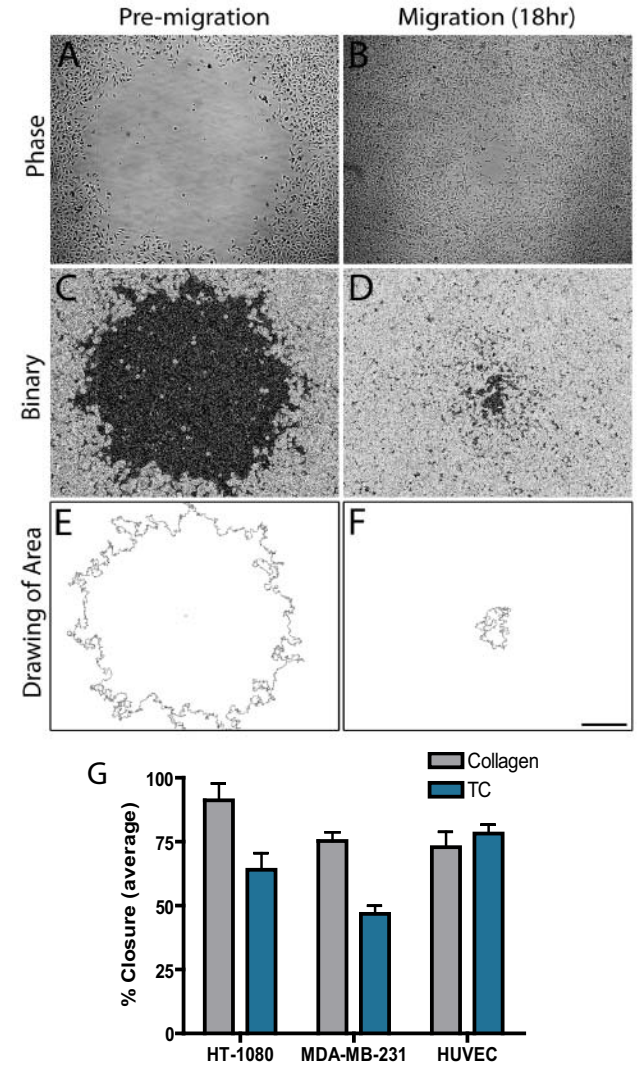

Figure 2. ImageJ analysis of cell migration by measuring area closure from phase-contrast images. A and B, Phase images of the pre-migration reference (A) and HT-1080 cells that have migrated on collagen I for 18 hours (B). C and D, Binary images created by ImageJ that correspond to the phase images in A and B. E and F, Area drawings traced from the corresponding binary images. Scale bar = 500µm. G, HT-1080, MDA-MB-231, and HUVEC migration presented as percent closure on both collagen I and TC surfaces. Data are presented as mean ± SD from 24 wells per condition.

Results continued...

Next, cell migration was calculated from area measurements of the Detection Zone obtained from fluorescence images. HUVECs were cultured on an Oris™ Pro TC plate. Following 24 hours migration in the presence of the MEK inhibitor, UO126, and the ROCK inhibitor, H-1152, cells were fixed and stained with TRITC-phalloidin. Representative fluorescence images (Fig 3A-D) and corresponding ImageJ area drawings (Fig 3E-H) demonstrate differences in migration in response to the inhibitors. Calculation of percent area closure using the areas of the pre-migration and migration time points demonstrate that HUVECs migrated to approximately 79% closure in control wells, while treatment with UO126 and H-1152 inhibited migration to 15% and 50% closure, respectively (Fig 3I).

Conclusions

This application note demonstrates a method to measure area closure of a cell monolayer in the Oris™ Pro Cell Migration Assay** by the use of ImageJ analysis software. Using this analysis method to automatically trace the Detection Zone and measure the area from phase-contrast images, the current study demonstrates HT-1080s, MDA-MB-231s, and HUVECs underwent varied amounts of migration on collagen I and TC-treated surfaces. The same analysis method was followed in ImageJ to analyze the Detection Zone based on fluorescence images. Additionally, the method was suitable for showing that HUVEC migration was inhibited by UO126 and H-1152 on TC treated surfaces. These results demonstrate that the area of the Detection Zone from phase-contrast and fluorescence images can be automatically traced and measured using ImageJ. As a result, cell migration can be accurately quantified by calculating percent closure of the Detection Zone in the Oris™ Pro Cell Migration Assay**.

**In addition to the Oris™ Pro Cell Migration Assay, analysis methods described in this application note can be used to quantify cell migration in the Oris™ Cell Migration Assay.

1. Horwitz R and Webb D, 2003. Curr Biol. 13(19):R756-R759.
2. Kees Straatman, 2008. www.le.ac.uk/biochem/microscopy/pdf/Wound%20healing%20assay.pdf.

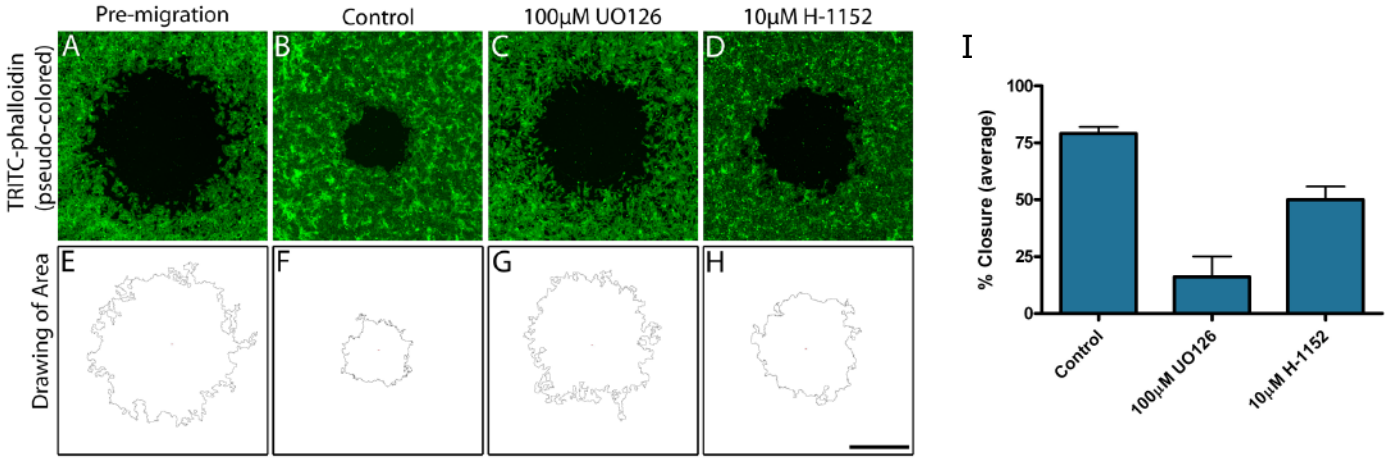

Figure 3. Effects of a MEK and ROCK inhibitor on HUVEC migration. A-D, Pseudo-colored images of a pre-migration well stained with TRITC-phalloidin, and cells that migrated for 24 hours on an Oris™ Pro TC treated plate in the presence of vehicle only (control), 100µM UO126, or 10µM H-1152. E-H, Corresponding area drawings created by ImageJ. Scale bar = 1000µm. I, Quantification of area closure calculated from measured areas at the pre-migration and migration time points. Data are presented as mean ± SD from 16 wells per condition.

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