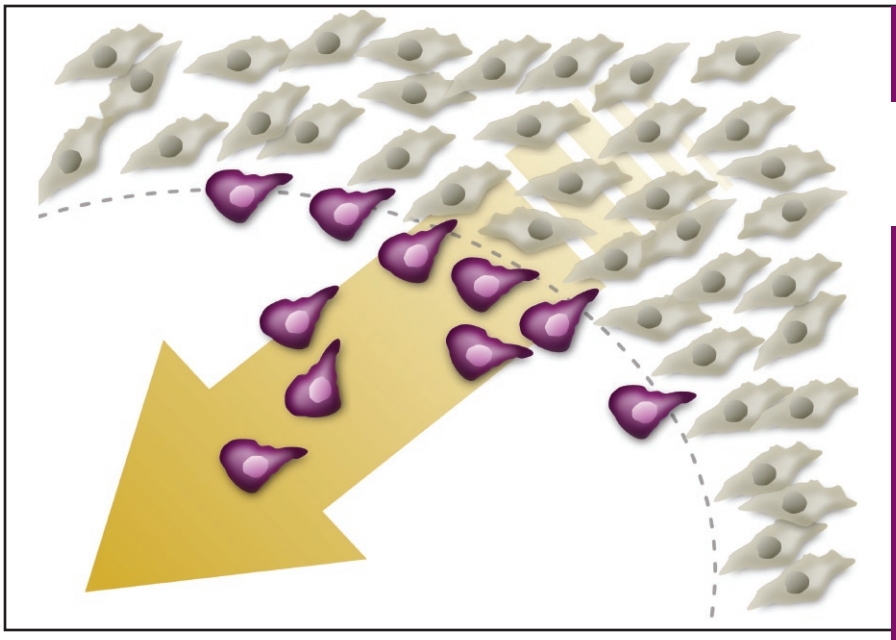

APPLICATION NOTE

Analysis of Cell Migration using the Operetta

Key Features

- Automated image acquisition of fixed cells using the Operetta™ System
- Data analysis using the flexible Harmony™ Software
- Image-based quantification of cell migration using the Oris™ Cell Migration Assay

Cell motility, Area analysis

Background

Cell migration plays a major role in a variety of physiological events, such as immune response and wound healing. In addition, cell migration can also contribute to pathological processes including metastasis [Lauffenburger *et al.*, 1996; Horwitz *et al.*, 2003]. Understanding the molecular components of migration is crucial for discovering new targets to develop drugs that affect migration. Identification of new therapeutics that manipulate cell migration would benefit from recent advancements in High Content Screening (HCS) technology.

Here, we present a method to analyze cell migration using the Operetta / Harmony HCS platform and the Oris™ Cell Migration Assay from Platypus Technologies, LLC. This sensitive and flexible assay is based on a 96-well format which provides robust and reproducible quantification of cell motility. An area-based approach was used to quantify the amount of migration.

Application

HT-1080 fibrosarcoma cells (3.5 x 10^4 cells / well) and MDA-MB-231 breast epithelial cells (3 x 10^4 cells / well) were plated on Oris™ Cell Migration Assay 96-well plates coated with Collagen I. Each well contained a silicone stopper that prevented cell attachment in the center region of the well. After allowing the cells to adhere to the surface for 6 hr (37 °C, 5% CO_2), half of the stoppers were removed to reveal a uniform 2 mm diameter pristine detection zone in the well (Figure 1) into which cells could then migrate. The remaining stoppers were kept in the wells to generate pre-migration controls as references. The medium was replaced and the cells were allowed to migrate for 18 hr. The stoppers were then removed from the pre-migration reference wells and cells in all wells were fixed (0.25 % glutaraldehyde, 15 min) and permeabilized (0.1 % Triton-X), followed by staining with TRITC-phalloidin (SigmaAldrich®).The images were acquired with the Operetta using a 10x high NA objective.

For image analysis, the Harmony **CellMigration-1** sequence was selected from the Ready-Made Solution collection. This module analysis strategy is based on open area quantification. The cell layer is separated from the background and quantified as open area by subtracting it from the whole image. Figure 2 shows the numerical output for the measured open detection zone before and after migration.

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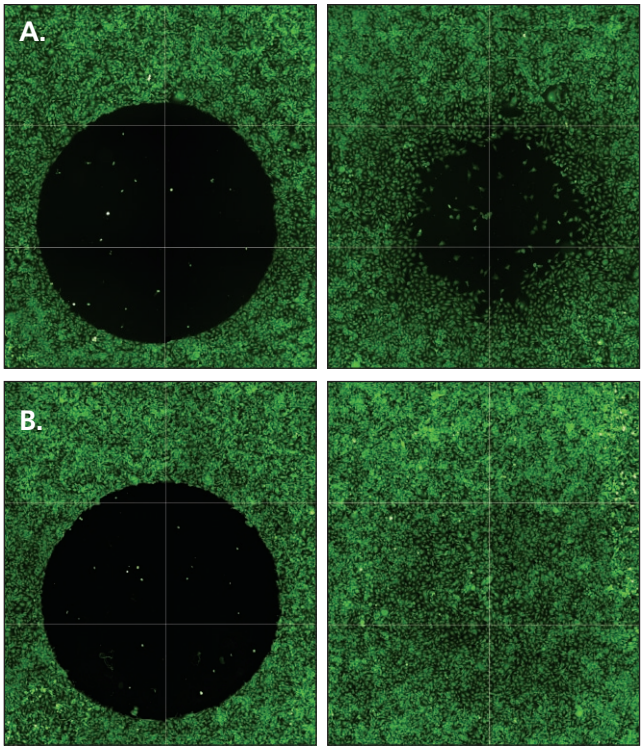

Figure 1. Pre-Migration and Post-Migration Image Analysis.
Images were captured using an Operetta 10x high NA objective. For illustrating the complete analytical zone 6 subfields were required. A | Well overview for MDA-MB-231 cells, showing a pre-migration control (left) and an area after 18 hr migration (right). B | Well overview for HT-1080 cells, showing a pre-migration control (left) and an area after 18 hr migration (right).

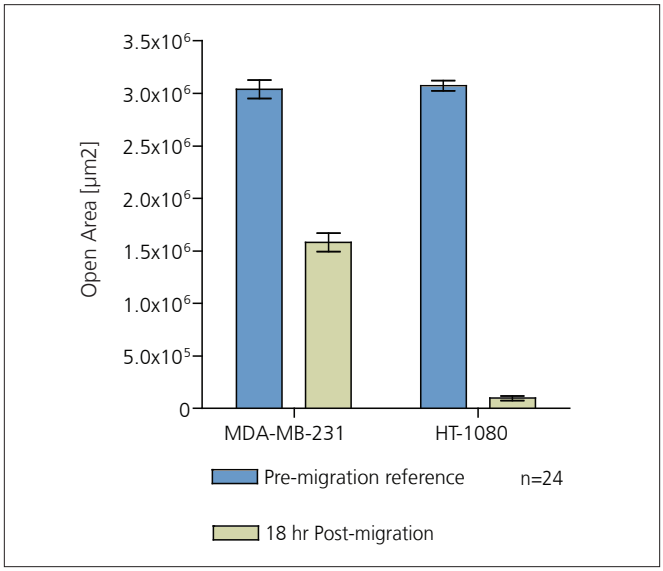

Figure 2. Quantitation of Cell Migration based on Open-Area Analysis.
Harmony software was used to calculate the open areas of MDA-MB-231 and HT-1080 cells pre- and post-migration. The values shown for the open areas are inversely proportional to the amount of cell migration within the Oris™ detection zone. Cells were seeded on the Oris™ assay plates, allowed to adhere for 6 hr and then half of the stoppers were removed. Following an 18 hr migration period, the remainder of the stoppers were removed to provide pre-migration controls. The open area within the pre-migration control wells for both cell lines (blue bars) corresponds to a consistently sized detection zone with a 2 mm diameter. The amount of open area remaining after 18 hr of migration was shown to differ between the 2 cell types (light green bars), indicating that in this experiment, HT-1080 cells migrate more than MDA-MB-231 cells on Collagen I. Data shown are the means for n=24 wells per group +/- SD.

Conclusions

Here, we present data acquisition and analysis from the Oris™ Cell Migration Assay using the Operetta / Harmony imaging platform. We demonstrate that our Harmony **CellMigration-1** analysis sequence provides robust and reliable results for the quantification of cell migration events.

The Oris™ Cell Migration Assay uses cell seeding stoppers to produce uniform detection zones for visualization of cell motility. The combination of the Oris™ Assay platform and the Operetta / Harmony system resolves many limitations associated with the classical *in vitro* scratch assay [Yarrow *et al*., 2004], such as high variability and cell disruption [Kam *et al*., 2008].

In addition to the area-based analysis highlighted here, the Harmony software provides the user with tools to evaluate single cell-based strategies.

References

Horwitz R, Webb D (2003): Cell Migration, Current Biology, 13 (19), R756-759

Kam Y, Guess C, Estrada L, Weidow B, Quaranta V (2008): A novel circular invasion assay mimics in vivo invasive behavior of cancer cell lines and distinguishes single-cell motility in vitro, BMC Cancer, 8, 198

Lauffenburger DA, Horwitz AF (1996): Cell migration: a physically integrated molecular process, Cell, 84 (3), 359-369

Yarrow JC, Perlman ZE, Westwood NJ, Mitchison TJ (2004): A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods, BMC Biotechnology, 4, 21

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