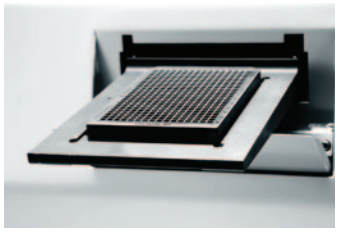


High-Throughput Cell Migration Assay

The biology of cell migration involves complex protein signaling mechanisms leading to changes in the cells cytoskeleton and ultimately to critical biological functions. Assays that measure cell migration enable a more detailed understanding of the mechanisms involved and are important for drug and therapeutic discovery efforts. One method commonly used to measure cell migration in the absence of a chemoattractant is the wound healing assay. This assay consists of scratched or "wounded" cell monolayers and there are many challenges associated with performing this assay in high-throughput fashion; one major problem being the lack of uniformity of the wound area. The Lin laboratory (Genentech) has demonstrated that cell migration in the PC-3 cell line is inhibited by specific drug and shRNA gene knockdown treatment. Here we report on the use of the **ImageXpress Velos System-DL™** (Blueshift Biotechnologies Inc.) laser scanning platform combined with the **Oris™ Cell Migration Assay** kit (Platypus Technologies). The kit format consists of a 96-well plate where cell monolayers are formed in an annular pattern in the presence of silicone stoppers that block attachment of seeded cells in the center of each well. After the stoppers are removed, cell migration into the previously blocked area is measured using the live cell fluorophore calcein-AM (Invitrogen) to label the cells for fluorescence detection. The **ImageXpress Velos System** scans whole well areas making it ideal for rapidly measuring cell migration within the uniform 2mm diameter unseeded (blocked) region in the center of the well. The results demonstrated an integrated image acquisition and image analysis process that enabled a simple, robust and high-throughput cell migration assay with the sensitivity required for the detection of shRNA or drug effects.

Assay Procedure

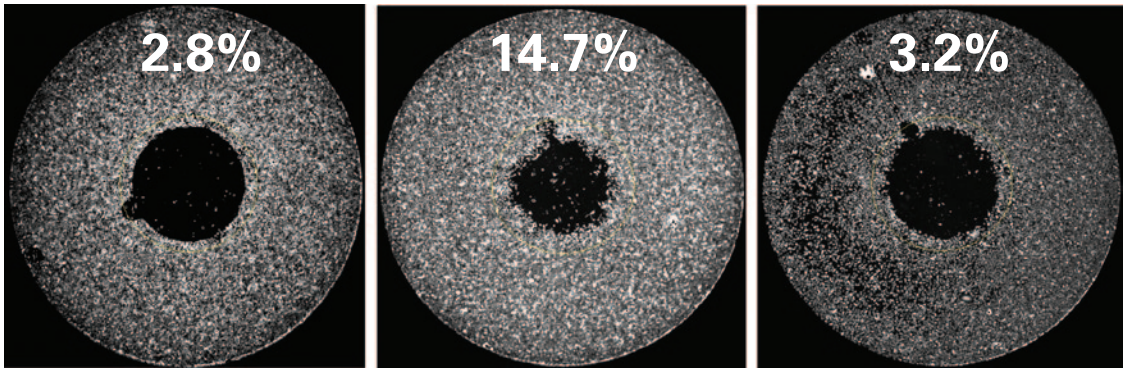
Cell Migration Assay. The cell migration assay using the **Oris™ Cell Migration Assay** kit (Platypus Technologies) was done in collaboration with Kui Lin's laboratory (Genentech) using the PC-3 cell model system for cell migration. Briefly, the cells were seeded at 15,000 cells/well and allowed to form monolayers. The cell seeding stoppers were removed from each well and the cells were washed once with PBS to remove any non-adhered cells. Media and/or drug agents were added and the cells were incubated for 24 hours. A calcein-AM solution (final concentration of 0.5 µM) prepared in PBS was added to label the cells for fluorescence imaging on the **ImageXpress Velos System**.

Fluorescence Imaging of Cell Migration. The **ImageXpress Velos System-DL** laser scanning platform used for this demonstration was configured with a 20mW 488nm laser. The 488nm laser was used for calcein-labeled cell detection using channel 1 (Ch1) 510-540nm band pass (bp) filter. The image acquisition was done at 5 x 5 micron sampling and an entire 96-well plate was scanned.

Results & Discussion

Cell Migration Imaging and Results

The **Oris™ Cell Migration Assay** is formatted for a 96-well black walled plate and uses stoppers made from medical grade silicone to restrict the cell seeding to the annular regions of the wells. Removal of the stoppers reveals a 2mm diameter unseeded region of each well into which the seeded cells may migrate (see **Figure 1**). An



Control 2 (C05) **shRNA2 - DOX (C09)** **shRNA2 + DOX (C10)**

Figure 1. Cell migration assay images for shRNA treatment versus a no migration control. The 2mm stoppers leaves a clean edge in the cell lawn, however nominal artifacts are observed in the unseeded region upon stopper removal. The shRNA2 treatment without DOX (C09) showed cell migration into the unseeded region as well as heavier cell growth. In contrast the DOX treatment with shRNA2 (C10) inhibited both cell migration and growth. The yellow circle corresponds to a 2.5mm diameter Region of Interest (ROI). The percent area coverage (Cell Area/ROI Area x 100) for all classified objects within a 2.1mm diameter ROI for each image is shown on the image (white font).

illustration of the **Oris™ Cell Migration Assay** is shown in **Figure 2** (provided by Platypus Technologies).

Oris™ Cell Migration Assay Procedure. Cells were treated with various agents for 24 h and stained with the live cell fluorophore calcein-AM. Cell migration was observed by using the **ImageXpress**

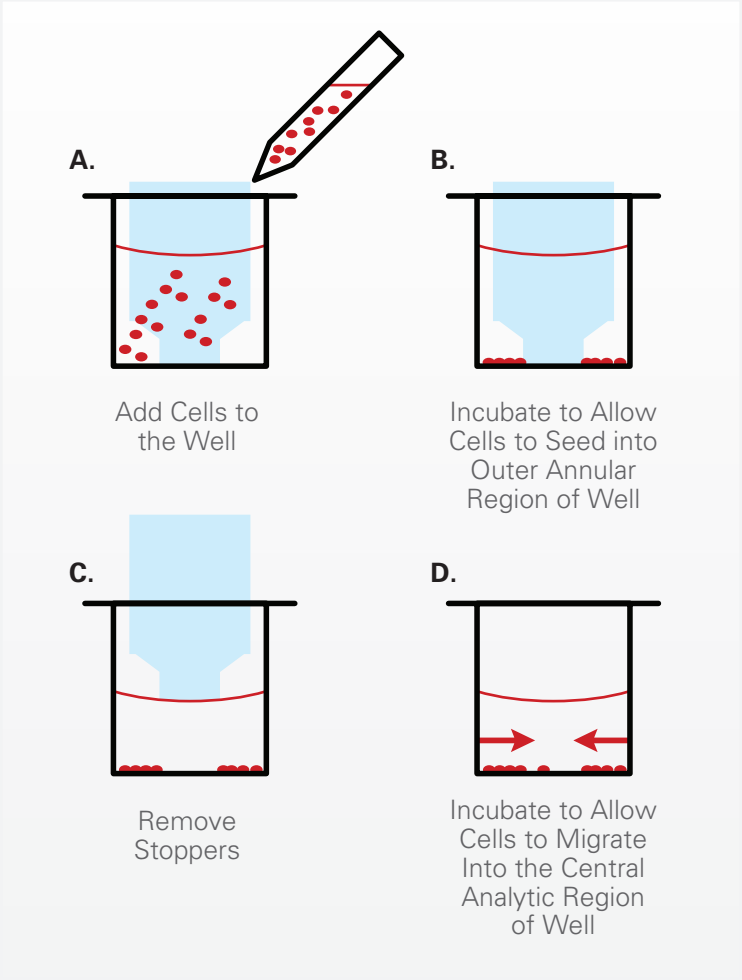


Figure 2. Illustration of Oris™ Cell Migration Assay procedure.

Velos System laser scanning platform to image the entire surface of each well. Green fluorescence from the calcein-AM labeled cells was acquired using a single channel of detection (Ch1) with a 510-540nm bp filter. A full 96-well plate is scanned and analyzed in under 5 minutes for the conditions used in this assay.

Whole well images of the calcein-labeled cells are shown in **Figure 1**. A control sample for no cell migration (Control 2) was obtained by removing the stoppers just prior to the calcein-labeling step. A nominal amount of cells are observed in the unseeded region of these control wells (see image of well C05 in **Figure 1**). All images were processed by an integrated image analysis program using a ROI of 2.1mm diameter and area filters to remove objects smaller than cells. The percent area coverage (Cell Area/ROI Area x 100) for all classified objects within the ROI for each image is shown on the image (white font).

Images of the effect of various shRNA treatments on cell migration are also shown in **Figure 1**. Differences were observed between the shRNA treated wells and the control wells. Using the **Oris™ Cell Migration Assay**, the ability of shRNA2 to block migration of PC-3 cells in the presence of doxycyclin (DOX) was demonstrated as shown in Figure 1 C10. The inhibition of migration (C10) is similar to the pre-migration image observed in the control wells where the stopper was removed at the end of the study just prior to staining (C05). In contrast, PC-3 cells were able to migrate when the cells were treated with shRNA2 and not induced with DOX (C09). These preliminary results indicate the ability of the **ImageXpress Velos System-DL** to successfully capture images for data analysis from a novel 96-well plate cell migration assay and warrant further optimization of this shRNA screening system in the Oris format. Optimization will include development of methods for the subtraction of cell background in the unseeded region, equal and optimized starting density of seeded cells, and optimizing the incubation time for optimal cell migration.

Conclusions

The **ImageXpress Velos System** provides a fast & simple acquisition and analysis process for the **Oris™ Cell Migration Assay**.

- Whole well images from a 96-well plate in < 5minutes
- Integrated image analysis to determine total cell area and percent coverage
- Higher data content and assay sensitivity available by whole-well imaging compared to plate readers
- Plate setup and sample manipulation is simple and easy to perform
- Currently available in 96 well formats

The unique optics and scanning engine of this platform enables simple "plug and play" applications to meet the needs of life science researchers in both academia and industry.

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