Oris™ Pro 384 Cell Migration Assays run on IN Cell Analyzer 6000

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Introduction

Cell migration and invasion both play important roles in many physiological and pathological processes such as wound healing and metastasis of cancer cells[1]. As these processes provide potential molecular targets for the development of novel therapeutics, there is significant effort to develop physiologically relevant assays suitable for high-throughput screening of compound libraries[2]. High-content imagers like GE Healthcare's IN Cell Analyzer 6000 are suitable instrumentation platforms for these applications by providing high-throughput multi-wavelength image acquisition at single-cell resolution, coupled with fast image analysis software, thus enabling collection of multiple end-point data in a single study[3].

The Oris™ Pro 384 Cell Migration Assay (Collagen I coated) from Platypus Technologies (www.platypustech.com) is a 384-well cell exclusion assay that may be useful for compound library screening. The assay plates are provided with spots of Biocompatible Gel (BCG) centrally deposited in each well to exclude cells from adhering in the centers of the wells. After the cells are seeded and allowed to adhere, the BCG self-dissolves to reveal reproducible cell-free Detection Zones in the center of each well, into which cells are then permitted to migrate (**Fig.1**). Cell movement is monitored in 2-Dimensions across the surface of the plate.

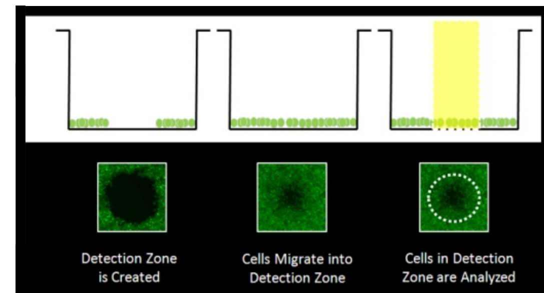

Figure. 1: Schematic of Oris™ Pro 384 Cell Migration Assay

Instrumentation

This study used the IN Cell Analyzer 6000, a laser-based line scanning confocal imager that uses sCMOS camera detection technology[4]. Unlike conventional point scanning confocal microscopy, the IN Cell Analyzer 6000 images the sample line-by-line, and instead of a physical barrier to block out-of-focus light, it uses the electronic rolling shutter of a CMOS camera as a virtual confocal slit. The sCMOS detector contains a 2560 x 2160 pixel array providing a large field of view (FOV) of 2.32mm². This makes the instrument suitable for the imaging of 2D migration assays since it allows single-field whole-well image capture from a 96-well plate with a 2x objective, and the same from a 384-well plate with a 4x objective.

We have previously studied[5] the use of the Oris™ Pro 96-Well Cell Migration Assay using the lamp-based IN Cell Analyzer 2000, which also utilizes a camera with a large FOV. This study uses the 384-well format of the assay and a new confocal imager from GE Healthcare.

Methods

Human umbilical vein endothelial (HUVEC) cells (ATCC) were seeded at 4,000 cells/well onto Collagen I coated Oris™ Pro 384 Cell Migration Assay plates in Medium 200 (Gibco). After 3hrs, media was removed and replaced with fresh medium containing 1/3 dilution series of Colcemid, Nocodazole, Paclitaxel and Colchicine (all from Sigma) and 2µM Cell Tracker™ Green (Invitrogen Molecular Probes). The cells were incubated for up to 48hrs followed by fixation for 1hr in 4% formalin containing 2.5µM Hoechst 33258 (Invitrogen Molecular Probes). Whole-well images were acquired with IN Cell Analyzer 6000 using a 4x 0.2NA objective using a 60msec exposure. Cell Tracker Green (FITC) Images were acquired as a time series after addition of the compounds (returning the plate to the incubator between images). After fixation of the cells, both Hoechst and FITC wavelengths were captured.

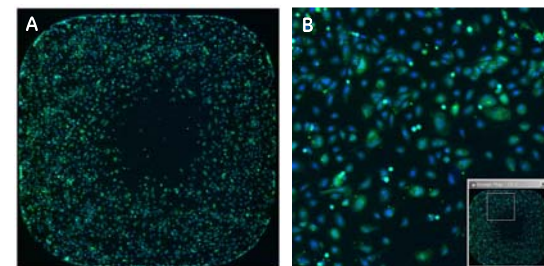

*Figure 2: **Example 4x single-field whole-well image from one well of the 384-well migration assay plate.** The Hoechst and Cell Tracker™ Green images are shown in a fused image display (**2A**). In **2B** the 4x image is digitally zoomed with IN Cell Investigator analysis software to display single-cell resolution.*

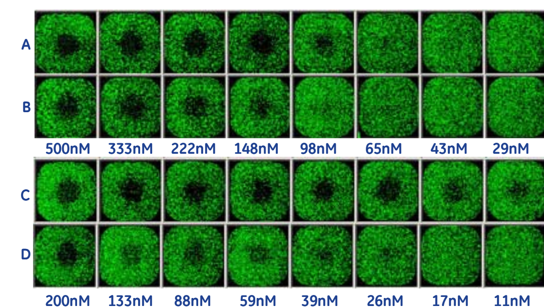

*Figure 3: **Thumbnail view images showing dose responses of the various drugs used in the study.** Example images from the titrations of Colcemid (**3A**) and Nocodazole (**3B**) are compared with treatments with Paclitaxel (**3C**) and Colchicine (**3D**). Images of the fixed cells after 48hrs of treatment are shown. The assay plate contained 4 replicate wells per treatment and the dose responses extended beyond the range shown in the figure.*

IN Cell Investigator software was used to analyze the images and data was collected for nuclear morphology and intensity from the Hoechst 33258 images, and from the Cell Tracker™ Green images the cell coverage per well was calculated.

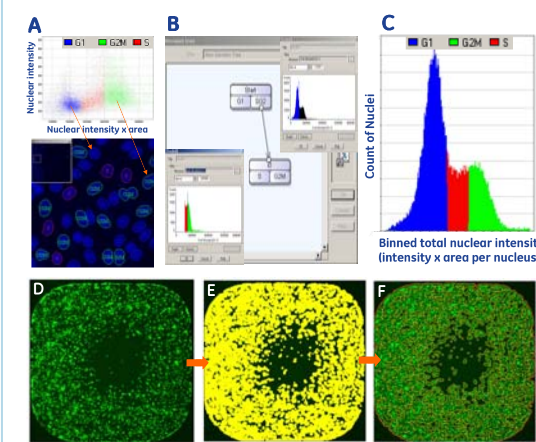

*Figure 4: **Image Analysis strategies using IN Cell Investigator.** Mean pixel intensity x area data per nucleus from the Hoechst 33258 images was used in a decision tree filter to classify cells into G1, S and G2M cell cycle phases (**4A-C**). To estimate cell coverage, Cell Tracker™ Green labelled cells (**4D**) were segmented (**4E**) and total cell area compared to the area measured for the whole well (**4F**).*

Results – End-Point Assay Cell Counts and Well Coverage

The drugs used all have known effects on the mammalian cell cycle by acting as mitotic spindle inhibitors. Nocodazole and Colchicine inhibit microtubule polymerization, Colcemid depolymerizes microtubules and Paclitaxel prevents microtubule disassembly. The analysis results looked at effects on cell migration and cell cycle phase, with cell health monitored by measuring nuclear morphology.

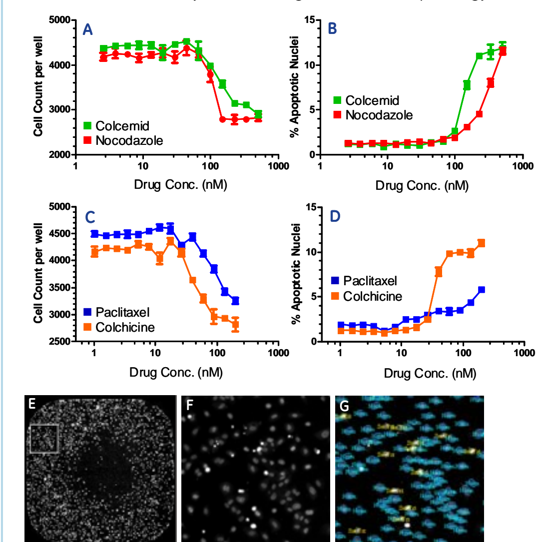

*Figure 5: **Results for cell counts and apoptotic nuclei per well.** The cell count data (**5A** and **5C**) is from the Hoechst image channel and % apoptotic nuclei (**5B** and **5D**) is also calculated from this wavelength using a classifier to identify nuclei with abnormally small area and bright intensity (**5E-5F**). These nuclei are indicated in yellow in **5G**. All data shown is +/- SEM (n=4 replicate wells per treatment). Both the apoptosis results and cell count data gave Z scores of >0.5.*

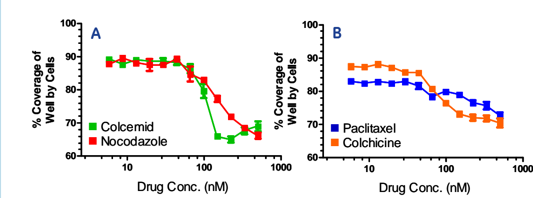

*Figure 6: **Coverage of the well by cells.** Cell coverage was calculated from the Cell Tracker™ Green images by comparing total area occupied with cells with the area of the well. All data shown is +/- SEM (n=4 replicate wells per treatment).*

Results – End-Point Assay Cell Cycle Classification

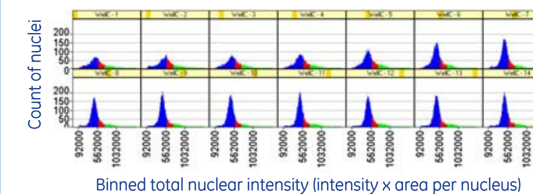

*Figure 7: **DNA content histograms for Colchicine-treated cells.** Spotfire trellis plots of binned Intensity x area per nucleus. The histograms are colored by cell cycle classification for G1 (blue) S (red) and G2M (green) cell cycle phase from IN Cell Investigator analysis data output. Data from 1/3 dilution series of Colchicine starting at 200nM in column 1 (designated well C1).*

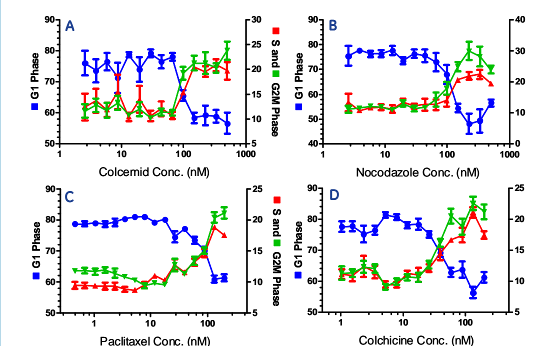

*Figure 8: **Cell Cycle Phase Classifications.** G1 (blue) S (red) and G2M (green) cell cycle phase classifications from IN Cell Investigator analysis of the Hoechst 33258-stained nuclei. All data shown is +/- SEM (n=4 replicate wells per treatment).*

Results – Kinetic Assay Cell Counts and Well Coverage

The drugs used were added to the cells in combination with a fluorescent non-toxic cell marker (Cell Tracker™ Green). Without washing the plates, which could lead to loss of loosely attached cells, the IN Cell Analyzer 6000 was used to obtain Cell Tracker™ Green images in wells containing both complete culture media and excess cell stain. Cell count and well coverage was calculated over the time course of the experiment. The plate was returned to the incubator between time points. The 4x objective allowed whole-well image capture (60msec exposure), and the read time per 384-well plate (ie per time point) was 11 minutes.

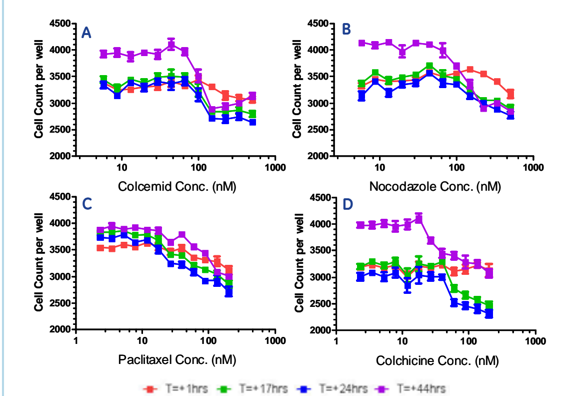

*Figure 9: **Cell counts over time course of the experiment.** Cell counts were calculated from IN Cell Investigator analysis of the Cell Tracker™ Green images. All data shown is +/- SEM (n=4 replicate wells per treatment).*

The kinetic analysis indicated that by 24hrs there was significant cell movement into the migration zone of the plates in control wells and wells with low concentrations of drugs (results not shown) but this was not accompanied with significant increases in cell numbers (**Fig.9**). However, during the first 24hrs of the experiment, measurable cell loss was apparent at the highest concentrations of Colcemid, Nocodazole and Colchicine. By 48hrs cell numbers were markedly increased in all wells except those with the highest concentrations of the drugs, with the exception of Paclitaxel. When comparing kinetic and end-point analysis, it is clear that both approaches provide valuable data on drug efficacy and mechanism of action, but kinetic study may offer more subtle mechanistic information useful to discriminate drug activity.

Conclusions

- **Use of the IN Cell Analyzer 6000 demonstrated for whole-well high-throughput imaging of cell migration using Oris™ Pro Cell Migration 384-well Assay plates.**
- **The assay delivers results with high Z scores (>0.5) and can be used for end-point and kinetic study of cell migration.**
- **Images acquired at 4x have sufficient contrast and resolution to allow analysis to single-cell level.**
- **The IN Cell Investigator analysis software was used to measure cell count and well coverage, and in addition provided data on nuclear morphology, allowing for classification of apoptotic phenotype and cell cycle classification.**

References

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