Measure cell migration using automated cell imaging and analysis

By Jayne Hesley[1], Rachel McMillan[2], Paula Gedraitis[1], and Michael Thomas[3]
[1] Molecular Devices LLC., San Jose, CA, [2]University of California Los Angeles , CA, [3]Platypus Technologies Inc., Madison, WI

INTRODUCTION

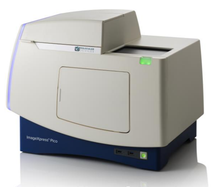
Cell migration, the relocation of cells, is relevant to wound healing, immunology, embryonic development, and irregular cellular events such as cancer metastasis. Cell migration assays are used to measure cell motility in a controlled environment, and both "scratch" assays and assays utilizing microplates with pre-formed "wounds" are frequently utilized for this purpose. By conducting the assay in a 96 or 384 well microplate, several compounds that may affect the cell's ability to migrate, such as chemotherapeutic compounds or drugs to speed up wound healing, may be tested in one controlled experiment with adequate replicates. The experiment may be acquired and analyzed in any of the ways outlined below.

Cell state	Assay type	Image format	Analysis
Live	Standard time-lapse	Transmitted Light or Fluorescence	On-the-fly or at end of time-lapse
Live	Discontinuous time-lapse	Transmitted Light or Fluorescence	On-the-fly or at end of time-lapse
Live or Dead	Endpoint	Transmitted Light or Fluorescence	On-the-fly or at end of experiment

We were able to successfully show the differences in wound healing of two cell types: HT1080 (fibrosarcoma) and U2OS (osteosarcoma) after treating with Cytochalasin D, Colchicine, Isoproterenol, Nocodazole, or Retinoic Acid, using either a manual scratch assay in a 96 well format or the Oris™ Pro Cell Migration Assay which utilizes a dissolvable biocompatible gel to create a cell-free zone into which the cells migrate.

MATERIALS AND METHODS

- Assay Reagents and Cells
  - U2 OS bone cancer cell line (Millipore Sigma P/N CLL1037)
  - HT 1080 fibrosarcoma cell line (ATCC P/N CCL-121)
  - SiR-Actin Kit (Cytoskeleton Inc. P/N CY-SC001)
  - Oris™ Pro Cell Migration Assay (Platypus Technologies P/N PRO384CMA1)
- Automated Imagers (Molecular Devices)



ImageXpress® Pico Automated Cell Imaging System with CellReporterXpress™ Software

ImageXpress® Micro High-Content Imaging System with MetaXpress® Software

DISCONTINUOUS TIME-LAPSE OF A SCRATCH ASSAY

U2OS cells were plated into 96 well microplates at 40,000 cells/well to form a confluent monolayer after growing overnight. To create a wound in each well, twelve 20 µL pipet tips were loaded onto a manual multi-channel pipettor and dragged firmly across each row of wells from top to bottom. Wells were then rinsed one time with 200 µL of warm DPBS before the addition of 200 µL of compounds prepared in media. The cell line was stably transfected to express RFP-actin. The plate was imaged, returned to the incubator and re-imaged periodically, appending to the previous reads to construct a "discontinuous" time-lapse data set.

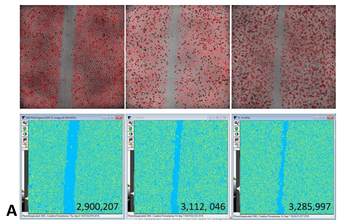


Figure 1. A. Top images show an overlay of U2OS cells acquired in both transmitted light and fluorescence (for Actin-RFP) at 1, 11, and 23 hours post-treatment with 0.1 µM colchicine. Directly below is the mask generated using a Transmitted Light Segmentation module in MetaXpress software. The yellow areas were identified as cells and the total area under the yellow mask is plotted in **B.** for Control and 2 compounds.

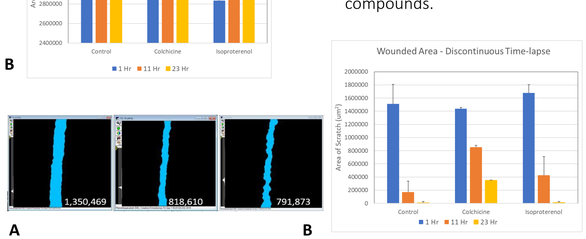
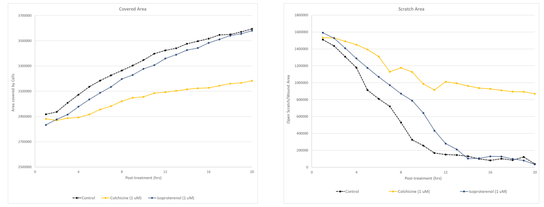


Figure 2. A. Transmitted Light images in Figure 1 were used to generate this light blue mask using a Custom Module to identify the scratch area that is free of cells at 1, 11, and 23 hours post-treatment with 0.1 µM colchicine. The masks clearly measure the decrease in scratch area over time as plotted in **B** along with Control and a less-inhibitory compound.

In a separate experiment, the plate of live cells remained inside the ImageXpress Micro system equipped with environmental control and was automatically imaged as a standard time-lapse assay.

Figure 3. Transmitted light images were acquired every hour and the scratch closure was measured by either "Area covered by cells" (left) or "Area of the Scratch" (right). A movie can be rendered of any well.



CELL MIGRATION ASSAY FOR HIGH THROUGHPUT

Cells were treated with the cell division inhibitor, Cytosine β-D-arabinofuranoside hydrochloride (Ara C), and 100 nM SiR-actin live cell stain, then plated in 384 well Oris Pro Cell Migration plates at 10,000 cells/well in 30 µL. After cells had attached and gel had dissolved for 2 hours, 30 µL of each compound was added to the wells. A discontinuous time-lapse series was collected using the ImageXpress Pico system over a 45-hour period. Cell migration was measured by calculating the area covered by cells in each well at each time point. Analyses protocols were optimized for both Transmitted light and fluorescent analysis and data can be generated simultaneously with acquisition.

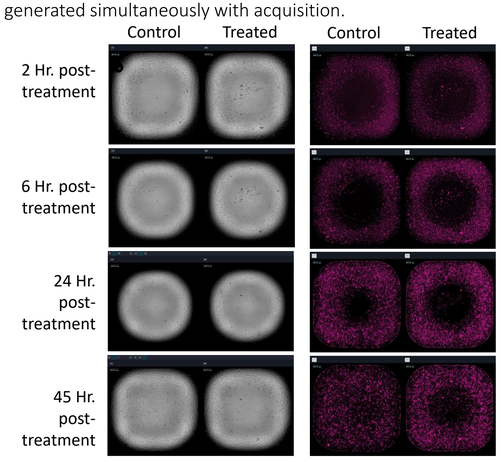


Figure 4. Example transmitted light and SiR-Actin images of an untreated HT1080 cell control well (left) compared to a well treated with 1 µM Nocodazole (right) at 4 representative timepoints. Nocodazole slowed migration into the exclusion zone.

COMPARABLE RESULTS WITHOUT FLUORESCENCE

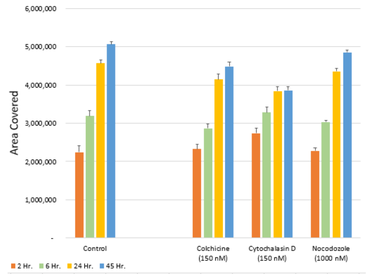


Figure 5. Analysis of fluorescently labeled cells over time demonstrates that compound-treated wells exhibited decreased cell migration.

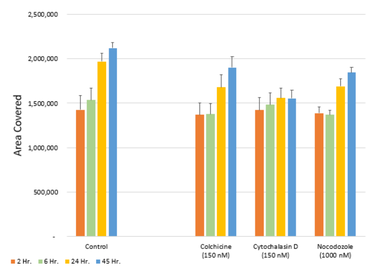


Figure 6. Transmitted light analysis over time demonstrates that compound-treated wells exhibited decreased cell migration.

CONCLUSIONS

- If using an ImageXpress system equipped with environmental control, plates may be read without user intervention at specified intervals.
- Discontinuous time-lapse assays reduce analysis time and storage space required for images while yielding meaningful cell migration results.
- The agreement between the transmitted light and fluorescent analyses indicate that cell migration assays may be run with or without fluorescent labeling.
- With the use of the Oris Pro 384 Cell Migration Assay, true screening for inhibitors of cell migration may be performed.

We would like to recognize Spirochrome Inc. and Platypus Technologies for their collaboration on this project.

SPIROCHROME Probes for Bioimaging

PLATYPUS TECHNOLOGIES

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