Application Note

Cell Migration and Invasion

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Automated High-Throughput Imaging and Analysis of Cell Migration

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Abstract

The ability of cells to migrate is a fundamental biological process in development and immunologic responses. It is also the defining feature of metastatic cancers. Therefore, it is important to measure the motile capacity of cells accurately and reliably. This application note introduces the Agilent BioTek Gen5 object tracking module, which automates analysis of 2D cell migration in high-content formats, including 96-well microplates. As opposed to manually tabulating cell movements one cell at a time under a single treatment condition, this feature can track thousands of cells across an array of conditions, such as a drug dilution series. These capabilities dramatically increase the efficiency and statistical robustness of drug screening applications.

Introduction

The concerted movement of cells within their environment plays a fundamental role in nearly all eukaryotic organisms. Higher eukaryotes rely on regulated cell migration throughout development and for immunologic processes, while aberrant cell migration is a defining feature of metastatic cancers. The process of cellular migration towards external stimuli, such as in chemotaxis, is called directional cell migration.[1-3] Equally as informative is random cell migration, in which cell trajectories are measured when a chemoattractant is diffused instead of emanating from a source direction. Whether studying directional or random migration, it is important to measure the motile capacity of cells accurately and reliably. In the simplest scenario, an object tracking algorithm and associated quantification capabilities should be able to distinguish between a control condition and a condition where the cells have been stimulated with a chemoattractant, or where their motile capacity has been disrupted.

The Gen5 object tracking module provides an automated method for characterizing 2D cell migration in high-content formats, including 96-well microplates. In contrast to manually tabulating cell movements one cell at a time under a single treatment condition, the Gen5 object tracking module can track thousands of cells across an array of conditions and treatments. Combined with Agilent BioTek automated imaging systems, this capability delivers an integrated solution for automating cell migration studies, increasing assay robustness and throughput.

This application note demonstrates the advantages of conducting cell migration studies with the Gen5 object tracking module in concert with the Agilent BioTek Lionheart FX automated microscope. Cytochalasin D (CytoD), a mycotoxin that targets and disrupts the normal function of the actin cytoskeleton required for cell motility, was used to quantify treatment-induced effects on cell migration within a microplate format.

Experimental

Cell lines

Immortalized human fibroblasts (CI-huFIB, part number INS-CI-1010) and human vascular endothelial cells (ci-huVEC, part number INS-CI-1002) were a kind gift from InSCREENex GmbH (Braunschweig, Germany). huFIBs were cultured in advanced DMEM (part number 12491; Gibco Thermo Fisher Scientific; Waltham, MA) containing 10% FBS and 1x penicillin/streptomycin/glutamine, whereas huVECs were maintained in HUVEC growth media: EBM-2 + EGM-2 BulletKit (part numbers CC-3156 and CC-3162, respectively; Lonza; Basel, Switzerland).

Fibronectin coating

Agilent 96-well imaging microplates (part number 204626) were treated with 10 µg/mL fibronectin (part number F1141; Sigma-Aldrich; Burlington, MA) diluted in DPBS for 30 minutes, followed by three washes with DPBS prior to cell seeding.

Cell seeding and drug treatment

Cells were pretreated with 0.5 µM Hoechst 34580 (part number H21486; Thermo Fisher Scientific; Waltham, MA) for two hours, then reseeded in 96-well plates at a density of 1×10^3 cells/well and allowed to attach to the fibronectin-coated well bottom at room temperature (RT) for 30 minutes. Once cells attached and started to spread, plates were incubated at 37 °C for at least two hours prior to drug treatment and kinetic imaging.

Kinetic imaging and image processing

Cells were imaged using a Lionheart FX fitted with a 4x 0.13 NA phase contrast objective, humidity stage insert, and set to 37 °C with 5% CO_2. Kinetic intervals were set to 10 minutes, with a total duration of 12 hours. Images were acquired in both phase contrast and DAPI channels, with the DAPI signal used to identify objects (nuclei), and the phase contrast channel used for laser autofocus as well as to kinetically align all frames of the run. A preprocessing background reduction step (30 µm rolling ball) was performed on the DAPI channel after kinetic alignment.

Object tracking—cellular analysis

Cell movement was measured by tracking their Hoechst 34580-stained nuclei; therefore, the DAPI channel was selected for the primary mask. A search radius was set to 50 µm (2x the nuclear diameter). The minimum life cycle of two frames was chosen to measure all possible objects, which was further refined to only those that were tracked for ≥ 6 hours through a subpopulation analysis step.

Results and discussion

The Gen5 object tracking module is a new tool that automates cell migration analysis.

Manually tracking objects, such as migrating cells, is a tedious process that involves frame-by-frame annotation. The Gen5 object tracking module not only automates object identification and tracking within kinetic image sets, but also enables those objects to be further analyzed with a subpopulation cellular analysis step. To illustrate this, a random migration kinetic run was performed in two wells of a 6-well culture dish using huFIBs pretreated with Hoechst 34580 to fluorescently label nuclei. Cell migration was inhibited in one well with 1 µM CytoD, while the other received vehicle control (DMSO). A kinetic run was then carried out on a Lionheart FX for 12 hours with 10-minute kinetic intervals at 4x magnification. Cell movement was then measured using nuclear signal (DAPI channel) to create a primary mask (Figure 1A). While all nuclei were initially identified, populations within the control and CytoD-treated wells were further refined to only those that were tracked for at least six hours (red), and only their tracks were displayed (Figure 1A). By automating this process with such a large image field, a large sample size can be measured. In this case, a median (M) velocity of 34.4 µm/h was measured for the control population with an n = 875, while the CytoD-treated population recorded a median velocity of 11.6 µm/h with an n = 977 (Figure 1B). A violin plot was chosen to represent this data because of its ability to concisely illustrate the velocity rate distribution of the population. To validate the accuracy of the Gen5 object tracking module, velocity rates were cross-checked and verified with ImageJ's TrackMate plug-in.

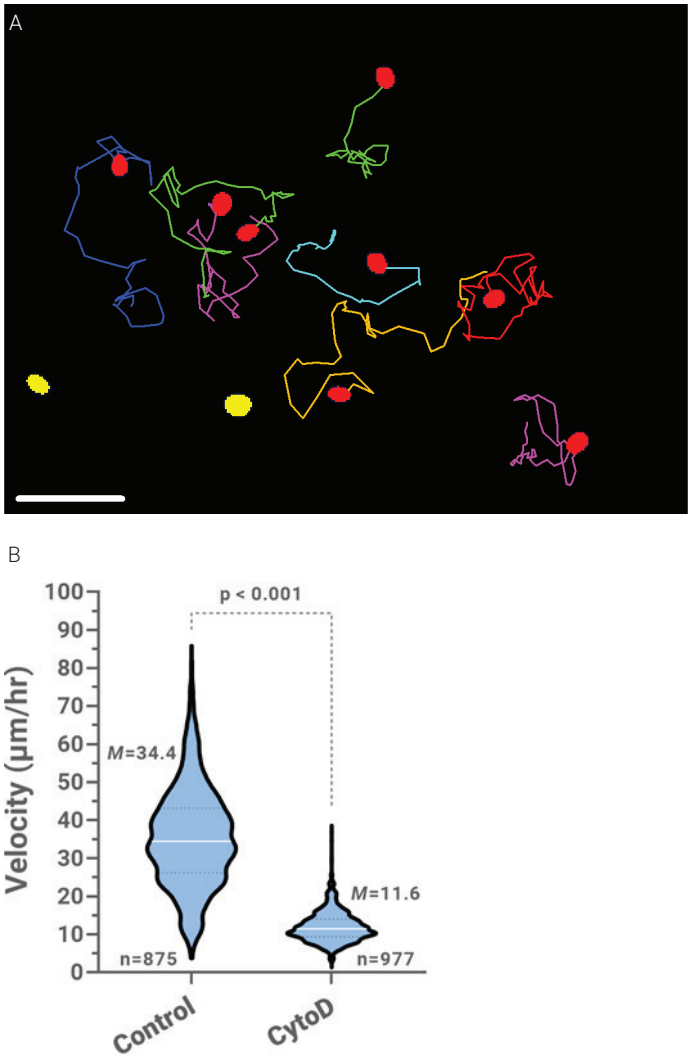

Figure 1. The Agilent BioTek Gen5 object tracking module automates cell migration analysis. A) huFIB cells were tracked by identifying their Hoechst 34580-labled nuclei for 12 hours. A subpopulation analysis refined the population of cells that were tracked for ≥ 6 hours (red) and whose tracks are displayed, while those cells that were not tracked for ≥ 6 hours are color coded in yellow. Scale bar = 100 µm. B) Violin plot illustrating the population-level velocity distribution of cells treated with 1 µM CytoD or vehicle control. The white solid line denotes the median velocity (M), while the grey dotted lines show the first quartile of the population.

The Gen5 object tracking module enables high-throughput analysis of cell migration.

While automated tracking and analysis of cell migration increases statistical robustness of individual samples, the ability to conduct batch analysis in a high-throughput format can provide insight to pharmacological studies that a single-sample analysis is simply unable to achieve. To demonstrate the power of the Gen5 object tracking module, a random migration assay was conducted in a 96-well microplate where ci-huVEC cells were treated with CytoD concentrations spanning four logs in 8-well replicates, with an n value of ≥ 700 cells tracked at each concentration (Figure 2).

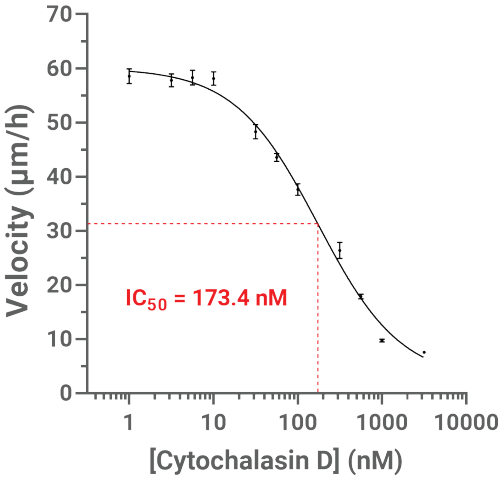

Figure 2. High-throughput migration assay establishes a CytoD IC_{50} for ci-huVECs. The Agilent BioTek Gen5 object tracking module can automate migration analysis in a 96-well microplate format, which enables an IC_{50} value of 175.4 nM to be derived based on a 12-point dilution series with 8-well replicates.

Mean velocities recorded for vehicle control (DMSO) were 57.7 µm/h, while CytoD at the high concentration used (3.16 µM) inhibited velocities down to 7.6 µm/h. Importantly, the capability to conduct migration studies in a high-throughput format enabled a dose–response IC_{50} to be derived, which was 173.4 nM (Figure 2). It should be noted that huFIBs and ci-huVECs were chosen for this study because they are not derived from a disease state and recapitulate wild type characteristics of their respective cell types.[4] However, the Gen5 object tracking module can be an important tool to study disease, such as cell types implicated in metastatic cancers.

Conclusion

The Agilent BioTek Gen5 object tracking module is a powerful tool that enables automated, high-throughput analysis of motile behavior, such as cell migration. Combined with the kinetic live cell imaging capabilities of the Agilent BioTek automated imaging systems, this powerful software feature provides an integrated platform for conducting a wide range of cell motility studies.

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