Application Note

Cell Migration and Invasion

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Automated Imaging and Analysis of 2D Chemotaxis

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Abstract

The ability of cells to migrate is a fundamental biological process in development and immunological responses. It is also the defining feature of metastatic cancers. The process of cellular motility towards external cues is collectively directional cell migration, for which migration towards a soluble chemical stimulant is referred to as chemotaxis. Measuring chemotaxis requires the ability to track cellular movement accurately and reliably. The Agilent BioTek Gen5 object tracking module combined with Agilent BioTek automated imaging systems delivers an integrated solution for automating cell migration studies, increasing assay robustness and throughput.

Introduction

The ability of cells to migrate is a fundamental biological process in development and immunological responses. It is also the defining feature of metastatic cancers. The process of cellular motility towards external cues is collectively termed directional cell migration, for which migration towards a soluble chemical stimulant is referred to as chemotaxis.[1] Measuring chemotaxis requires the ability to track cellular movement accurately and reliably. Informative metrics that evaluate cell motility are: A) velocity; B) Euclidean distance; C) total distance traveled; and D) X and Y displacement. From these measurements it is possible to derive metrics that give deeper insight into directional cell migration, such as the forward migration index (FMI).[2,3] This study describes the Gen5 object tracking module, which automates chemotaxis analysis.

Experimental

Cell lines

HT-1080 fibrosarcoma cells were purchased from ATCC (part number CCL-121; Manassas, VA) and were cultured in advanced DMEM (part number 12491; Gibco Thermo Fisher Scientific; Waltham, MA) containing 10% FBS and 1x penicillin/streptomycin/glutamine.

ibidi µ-Slide Chemotaxis setup

µ-Slide Chemotaxis chambers (part number 803626; ibidi; Gräfelfing, Germany) were set up according to the manufacturer's instructions (Figure 1A). Chambers C/D and E/F were plugged prior to flowing 12 µL of 10 µg/mL fibronectin (part number F1141; Sigma-Aldrich; Burlington, MA) through channel A/B. The channel was incubated at room temperature (RT) for 30 minutes followed by three washes with culture media. Cells pretreated with 0.5 µM Hoechst 34580 for two hours were passaged and resuspended to 2×10^6 cells/mL in media containing 10% FBS. They were then flowed through channel A/B, and allowed to settle and reattach for one hour at RT. Once attachment and spreading became apparent, media was exchanged with serum-free media.

Channel A/B ports were then plugged, and cells underwent a four-hour serum-starvation period at 37 °C. Following the serum-starvation period, chamber C/D was filled with 75 µL of serum-free media (sink), while chamber E/F was filled with media containing 10% FBS. The µ-Slide was then mounted on an Agilent BioTek Lionheart FX automated microscope with humidity stage and allowed to equilibrate for two hours prior to imaging.

Kinetic imaging and image processing

Cells were imaged using a Lionheart FX fitted with a 10x 0.3 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 average nuclear diameter). The minimum life cycle of two frames was chosen to identify all possible objects, which was further refined to only those that were tracked for six hours by including a subpopulation analysis. FMI in both the direction of the gradient ($FMI_{//}$) and perpendicular to the gradient ($FMI_{\perp}$) was calculated as a custom metric as follows:

$$FMI = (x_{last} - x_{first}) / (\text{Euclidean distance})$$

Results and discussion

The ibidi µ-Slide Chemotaxis is compatible with the Lionheart FX

To track chemotactic movement, it is important that the imaging area between channel A/B can be viewed (Figure 1A). To accomplish this, a custom plate layout was designed using a two-slide slide holder such that two µ-Slide Chemotaxis slides could be mounted at a time, enabling six conditions to be set up. A 10x objective is sufficient to capture the entire width of the chamber (1 mm) in wide field of view mode (Figure 1B). When chambers E/F are filled with 100% chemoattractant, and chambers C/D filled with media containing 0% chemoattractant (sink), a diffusion gradient is formed across the imaging area in channel A/B (Figure 1A). To verify that a gradient was formed, chamber E/F was filled with 50 µg/mL TRITC-dextran (40 kDa), and the formation of a gradient was monitored over several hours via line-scan quantification of the box in Figure 1B. A gradient formed almost immediately, stabilized within two hours, and it remained stable for up to 18 hours (Figure 1C).

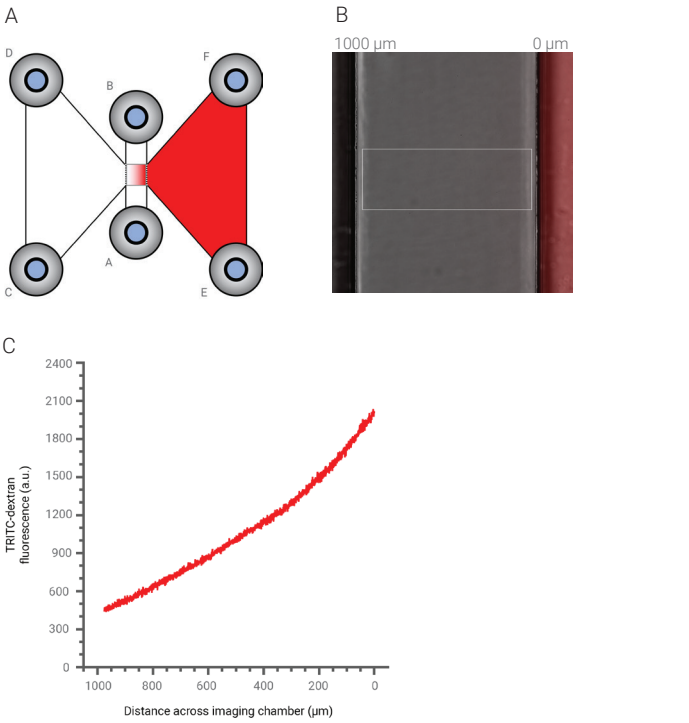

Figure 1. Establishing a gradient in an ibidi µ-Slide Chemotaxis chamber. A) Illustration of the ibidi µ-Slide Chemotaxis chamber. Cells are seeded in the imaging channel between ports A and B, whereas a chemoattractant can be added into the E/F chamber, while the C/D chamber is filled with media lacking chemoattractant. A gradient is then formed across the A/B imaging area. B) The 1 mm imaging area of the A/B channel taken with a 10x objective. Shown are both phase contrast and TRITC channels. The E/F chamber was loaded with TRITC-dextran. TRITC-dextran signal was measured with a line scan spanning the length and width of the white box, and reported in (C).

The Gen5 object tracking module enables trajectory quantification, including forward migration index

The Gen5 object tracking module is capable of reliably tracking objects over time (Figure 2), which provides trajectory metrics, such as displacement and velocity. An insightful measure of directed cell migration is FMI, which takes into account the displacement (Euclidean distance) of an object as it relates to the source direction of the chemoattractant gradient. The chemotactic capacity of HT-1080 cells was determined by tracking cells as they migrated through a gradient of FBS in a µ-Slide Chemotaxis chamber and deriving both the $FMI_{//}$ and $FMI_{\perp}$ (Figure 3). Additional trajectory metrics reported are displacement and velocity (Table 1).

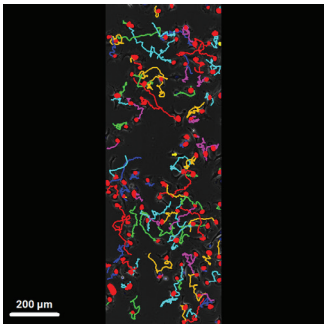

Figure 2. Cells were identified with a primary mask using their fluorescently labeled nuclei, and their movement tracked (multicolor traces) and analyzed with the Agilent BioTek Gen5 object tracking module.

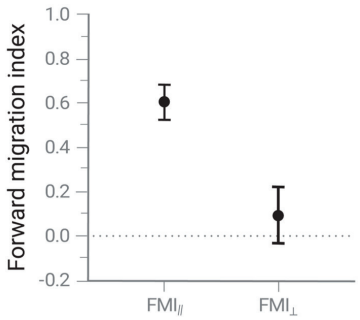

Figure 3. FMI of HT-1080 cells migrating in an FBS gradient was determined in the direction of the gradient ($FMI_{//}$) and perpendicular to the gradient ($FMI_{\perp}$).

Table 1. Insightful metrics to assess directed cell migration.

	Control
$FMI_{//}$	0.607
$FMI_{\perp}$	0.098
Euclidean distance	127.5
Velocity (µm/h)	34.1

Conclusion

The ability of cells to migrate towards chemical cues is a fundamental biological process. Forward migration index is a measure of a cell's ability to migrate towards a stimulus. The Agilent BioTek Gen5 object tracking module automates quantification of directional cell migration, such as chemotaxis. Combined with Agilent BioTek automated imaging systems, it delivers an integrated solution for automating cell migration studies, increasing assay robustness and throughput.

References

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