Technical Overview

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Agilent BioTek Gen5 Object Tracking Module: Tips and Tricks

Introduction

The ability of cells to migrate is fundamental for many biological processes and is also the defining feature of metastatic cancers. When evaluating the motile capacity of cells, it is important that their movement is recorded accurately and reliably. The Agilent BioTek Gen5 object tracking module automates analysis of 2D cell migration, whether it is random migration, or directed migration such as chemotaxis. When planning an imaging session for the purpose of tracking cell movement, there are a few aspects to consider, which are detailed below.

Optimize culture conditions and experimental design

- *Seed cells on an extracellular matrix (ECM)-coated vessel*. While the choice of ECM (and its concentration) largely depends on the cell type and integrin expression profile, cells will display a higher degree of motility when adhered to an ECM compared to the bare culture material, such as glass or tissue culture-treated plastic.
- *Optimize cell seeding density*. One can imagine that a cell can only reach its maximal motility rates when it is unobstructed by another object in its path. Additionally, it has be argued that once a cell comes up and interacts with another cell, its trajectory has inherently been altered and subsequent kinetic data is invalid. Therefore, choosing a seeding density should be influenced by the following: A) the lag time between seeding and experiment execution; B) the motility rates of the cells; and C) the duration of the kinetic run.

– *Choose the right fluorescent marker to identify objects*. Using a fluorescent marker will provide the best possible contrast, which in turn ensures cells can be reliably identified and tracked. However, employing fluorescent markers when conducting live imaging studies has its own challenges, such as introducing cyto- and photo-toxic effects. For example, Hoechst 34580 is an excellent nuclear marker for live cell imaging and tracking cell movement, but in moderate to high concentrations (> 1 µM), it can be cytotoxic. Additionally, iterative exposure to the UV excitation light needed for Hoechst signal detection can have a phototoxic effect that influences cellular behavior, such as motility. Alternative fluorescent markers are cell-defining dyes, such as CellTracker or CellMask.

Choose an appropriate kinetic interval

Any tracking algorithm, the Gen5 object tracking module included, works best when an object's X,Y position overlaps between adjacent kinetic frames. However, frequent collection of frames (oversampling) may ensure reliable tracking at the cost of an unnecessarily increased data set size. Choosing the appropriate kinetic interval largely depends on the cell type and culture conditions and must be established empirically. The Gen5 object tracking module maintains an object's identity by performing a radial search from the previous frame and identifies the nearest object in the subsequent frame and the same object. This search radius can be adjusted in the event the kinetic interval is too frequent or too long.

Include an auxiliary transmitted light channel for kinetic alignments

Imaging multiple areas, whether in a multiwell format or montaging, means that stage movement will occur. Such movement introduces variability to the true change in X,Y position from frame to frame of the object being tracked. Because of the increased pixel density, this variability per pixel is more pronounced at higher magnifications (40x and above). Ultimately, this variability can dramatically affect trajectory metrics of an object. In particular, image montages that require stitching can introduce detrimental effects to trajectory data if there is not enough structural information to align and stitch montages accurately.

Independent of the Gen5 object tracking module, Agilent BioTek Gen5 microplate reader and imager software can kinetically align images. However, by the very nature of measuring moving objects, the channel used for object tracking analysis should not be used to perform a kinetic alignment step. Instead, use a channel that contains nonbiological structural features that can be used as reliable markers. A convenient and effective technique for aligning images for object tracking applications utilizes a subtle crosshatch pattern on the Agilent 96-well imaging microplate bottom (part number 204626). While this pattern is not apparent during normal imaging applications, it can be captured using slightly defocused brightfield or phase contrast imaging (Figure 1). This kind of structural information will give the best results for aligning kinetic data.

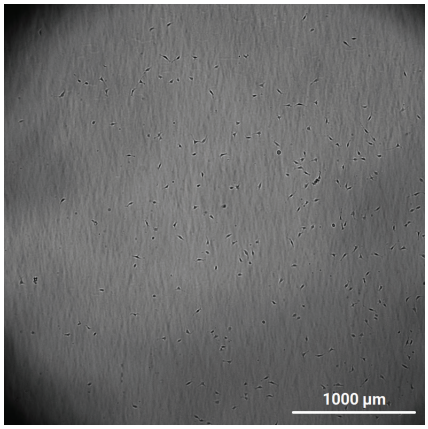

Figure 1. Crosshatch patterning of the well bottom of the Agilent 96-well imaging plate imaged at 4x with defocused brightfield, which can be used to maximize kinetic alignment accuracy.

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