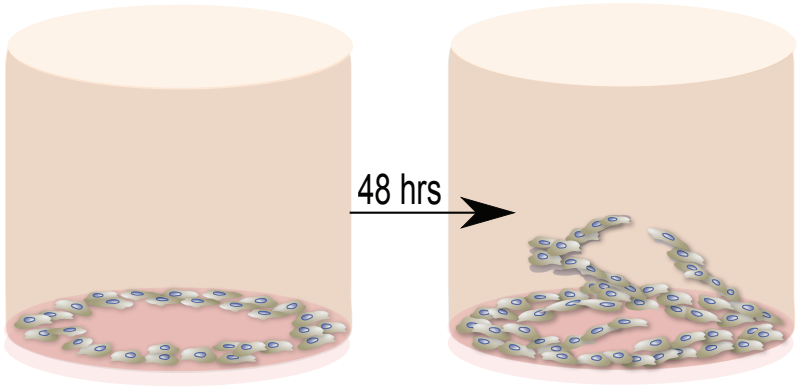

APPLICATION NOTE

3D Analysis of Cell Invasion using Operetta

Key Features

- Confocal image acquisition in three dimensions (XYZ)
- Quantification of cells in a 3D matrix using the Oris™ Invasion Assay
- Multiple readouts for inhibition of cell invasion: cell counting and morphological analysis of cell invasion modes

Cell Counting & Morphological Analysis

Background

Cell invasion is of major importance in many biological processes such as embryonic development, inflammatory responses, the repair of injured tissues, and tumor formation and metastasis [Horwitz and Webb, 2003]. During metastasis, individual cancer cells leave the initial tumor mass, enter the circulatory system and move into distinct parts of the body [Lauffenburger and Horwitz, 1996]. In order to successfully invade *in vivo*, metastatic cells must first permeate the basal lamina barrier by enzymatic degradation of extracellular matrix proteins before entering neighboring tissues [Kam et al., 2008]. Understanding the molecular mechanisms underlying tumor cell motility through 3D tissue environments is crucial for identifying new therapeutic targets for cancer prevention and treatment [Carragher, 2009; Kam *et al*., 2008].

Here, we present a method for analyzing cell invasion into a 3D extracellular matrix using the Operetta® / Harmony® High Content Screening (HCS) Platform and the Oris™ Cell Invasion Assay from Platypus Technologies, LLC. The confocal image acquisition performed by the Operetta High Content Screening System allows robust and reliable cell counting within different planes of the matrix. Additional morphological readouts help to distinguish between collective and individual cell invasion modes.

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Application

Oris™ Cell Invasion Assay 96-well plates were coated with basement membrane extract (BME) from the Murine Engelbreth-Holm-Swarm tumor. HT-1080 fibrosarcoma cells (3.5 x 10^4 cells / well) were then seeded onto the prepared plates. Each well contained a silicone Oris™ Cell Seeding Stopper to prevent cell attachment in the center region of the well (detection zone). After allowing the cells to adhere for 2 hr (37 °C, 5 % CO_2), the stoppers and media were removed and a 3D overlay was created by adding 40 µl of 10 mg/ml BME solution to each well. Stoppers were allowed to remain in designated wells until the end of the experiment to serve as pre-invasion references. To enable polymerization of the 3D overlay, plates were incubated for 1 hr at 37 °C and then fresh media containing various concentrations of the actin polymerization inhibitor, cytochalasin D, were added. Cells were allowed to invade the BME gel for 48 hr, and were then fixed (3.7 % formaldehyde), permeabilized (0.5 % Triton) and stained with Hoechst 33342 and rhodamine phalloidin (Invitrogen®). Three planes (0 µm, +15 µm, +30 µm) of confocal images were acquired with Operetta using the 10X high NA objective (Figure 1).

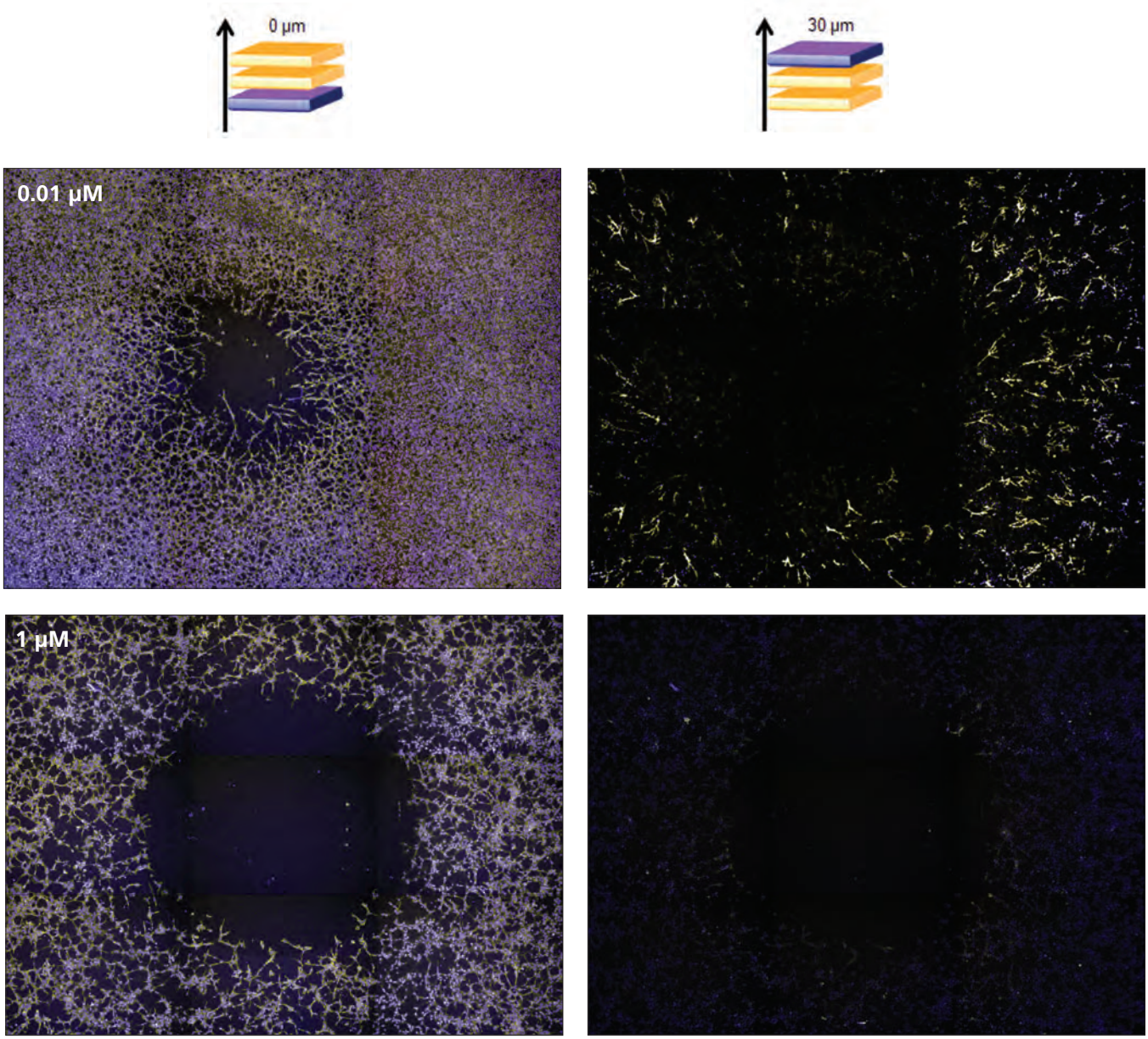

Figure 1. Well overviews showing cell invasion of HT-1080 cells in plane 1 (0 µm, left column) and plane 3 (30 µm, right column). Confocal images were captured using the Operetta 10X high NA objective. At low cytochalasin D concentrations (0.01 µM), cells migrated into the detection zone created by the stoppers (plane 1) and invaded the BME network in 3D (plane 3). High cytochalasin D concentrations (1 µM) inhibited cell motility in 2D and 3D.

The strategy for cell invasion analysis with the Harmony software is based on counting nuclei in the different planes. Nuclei were assigned to a specific plane by setting intensity thresholds for Hoechst staining and removing nuclei from other planes which appear as residual dim signals. As compounds like cytochalasin D may not only affect the invasiveness of cells but also the cell proliferation, results were normalized by calculating the ratio of detected nuclei in a specific plane to the number of detected nuclei in all planes. Invasion could then be plotted as the percentage of cells in plane 1, 2 and 3 (Figure 2).

Cell morphology readouts were generated using the "Find Nuclei" image analysis building block in the Harmony software to define specific collective cell invasion clusters as objects. By calculating the rhodamine phalloidin intensity and introducing a threshold (in this case signal intensity > 720), faint objects from other focal planes were excluded from the analysis. The "Select Region" building block was used to generate a skeleton for each object. As the skeleton area represents the amount of cell cluster branching, it is a good measure for the real object dimensions (Figure 3).

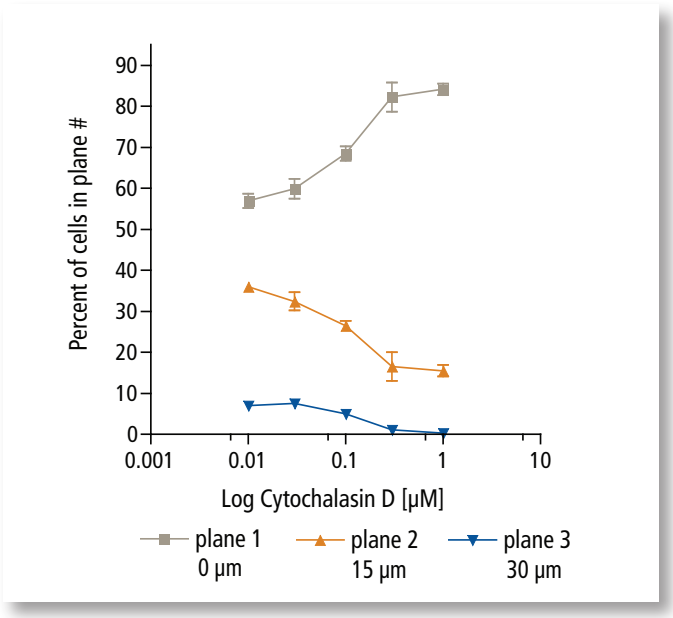

Figure 2. Inhibition of cell invasion by cytochalasin D. The relative number of cells in plane 2 (15 µm) and plane 3 (30 µm) decreases in a dose dependent manner, while plane 1 (0 µm) shows an increase. Each data point represents 3 wells.

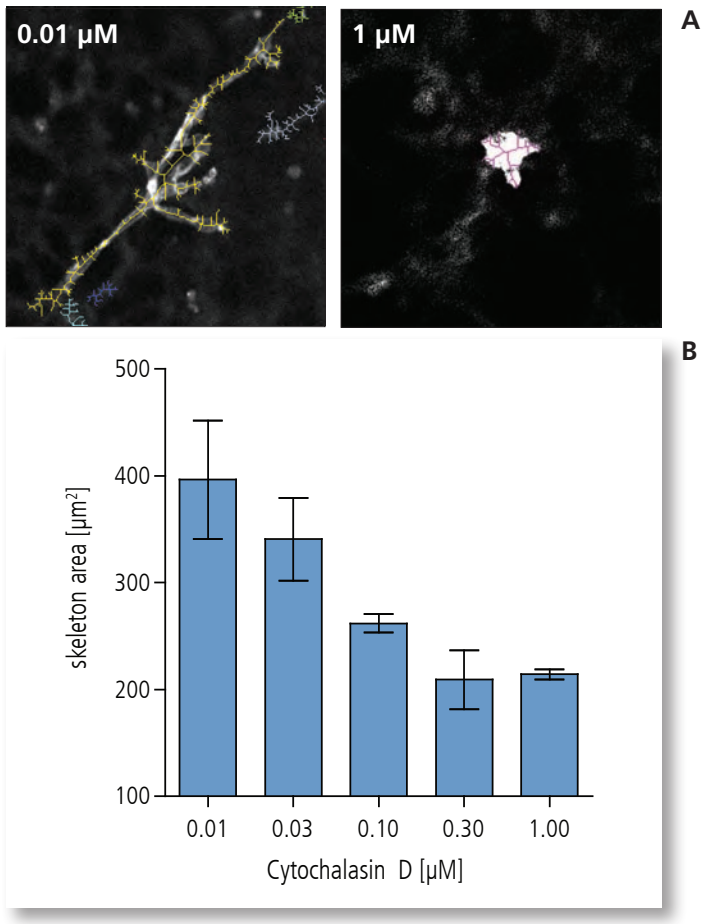

Figure 3. Morphological analysis of invasion mode.

A | Collective multicellular invasion clusters of 30 µm in height above the well bottom were defined as objects and object skeletons were generated. The skeleton serves as a measure of the length and extent of branching of invading cells.

B | Quantification of the skeleton area shows decreasing values as cytochalasin D concentrations increase, indicating a transition from a collective to an individual invasion mode. Each data point represents 3 wells.

Conclusions

In this study, we analyzed a dose dependent inhibition of cancer cell invasion into a 3D extracellular matrix. We demonstrate that the confocal image acquisition performed on Operetta and the image analysis using the Harmony software leads to a robust and reliable quantification of cell invasion. Additional morphological readouts provided insight into collective or individual cell motility.

The Oris™ Cell Invasion Assay is a high-throughput compatible invasion assay that overcomes the drawbacks of classical transmembrane based invasion assays. Using a 3D matrix overlay, cell invasion can be analyzed in a more biologically relevant invasion environment with a higher reproducibility [Kam *et al*., 2008]. In contrast to being limited to area-based readouts such as the degree of cell confluency or the wound width in scratch wound assays that reflect whole cell population dynamics, Harmony enables the analysis of single cell morphologies and therefore the distinction of different invasion modes.

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