Measuring of migration in a fully automated imaging based approach

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1 Introduction

Cell migration plays a major role in a variety of physiological events, such as immune response and wound healing. It also contributes to pathological processes such as metastasis. Understanding the molecular components of migration is crucial for discovering new targets to develop drugs that affect migration. Conventional technologies, e.g., transmembrane insert-based assays, used for measuring cell migration are not suitable for automation. High throughput measurements that can identify new therapeutics for modulating cell migration would benefit from high density plate format approaches and from recent advancements in image based High Content Screening (HCS) technology.
Here, we present imaging based measurement of wound healing and cell invasion.

2 Wound healing assay

HT-1080 cells (3.5E4 / well) and MDA-MB-231 cells (3E4 / well) were plated on Oris™ Cell Migration Assay 96-well plates coated with Collagen I. Each well contained a silicone stopper that prevented cell attachment in the center region of the well. After allowing the cells to adhere to the surface for 6 hr (37 °C, 5% CO2), stoppers were removed to reveal a uniform 2 mm diameter Detection Zone in the well (Figure 1) into which cells could then migrate. To generate pre-migration references some stoppers were kept in the wells until fixation occurred. The medium was replaced and the cells were allowed to migrate for 18 hr. The stoppers were then removed from the pre-migration reference wells and cells in all wells were fixed (0.25 % glutaraldehyde, 15 min) and permeabilized (0.1 % Triton-X), followed by staining with TRITC-phalloidin (SigmaAldrich®).

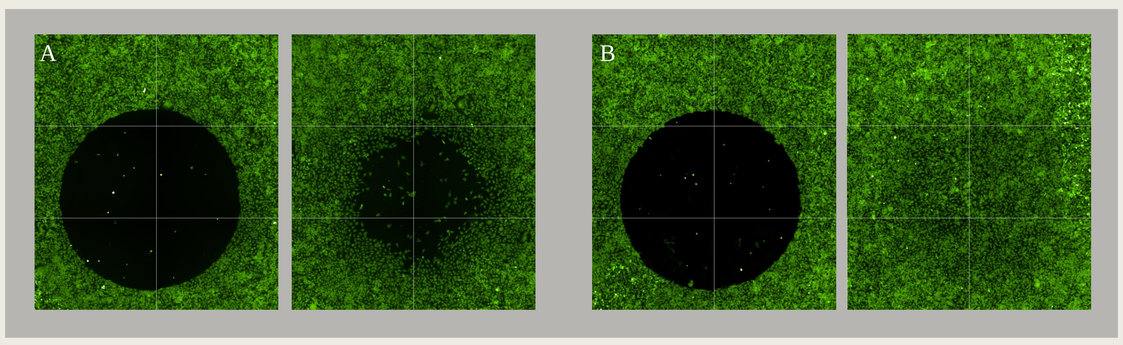

Figure 1. Pre-Migration and Post-Migration Image Analysis. Images were captured using an Operetta 10x high NA objective. For illustrating the complete analytical zone 6 subfields were required. A | Well overview for MDA-MB-231 cells, showing a pre-migration control (left) and an area after 18 hr migration (right). B | Well overview for HT-1080 cells, showing a pre-migration control (left) and an area after 18 hr migration (right).

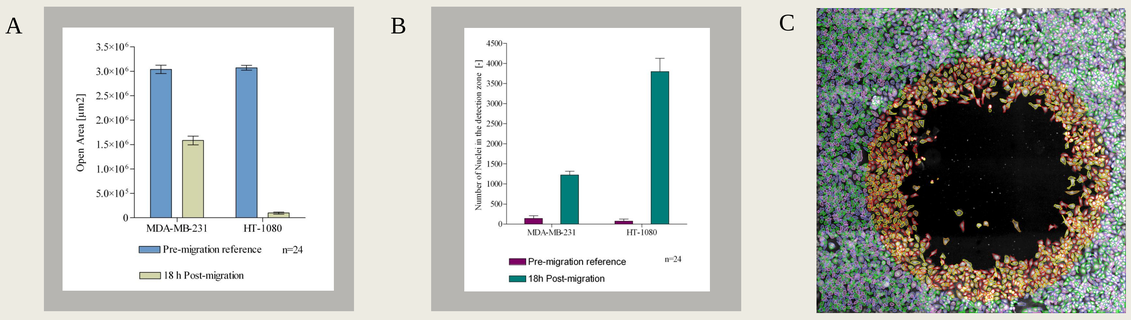

Figure 2. A | Quantitation of Cell Migration based on Open-Area Analysis. The values shown for the open areas are inversely proportional to the amount of cell migration within the Oris™ Detection Zone. The open area within the pre-migration control wells for both cell lines (blue bars) corresponds to a consistently sized Detection Zone with a 2 mm diameter. The amount of open area remaining after 18 hr of migration was shown to differ between the 2 cell types (light green bars), indicating that in this experiment, HT-1080 cells migrate more than MDA-MB-231 cells on Collagen I. Data shown are the means for n=24 wells per group +/- SD. B | Quantification of Cell Migration by counting the nuclei in the Detection Zone. The nuclei were counted only in the Detection Zone by creating a *region-of-interest* (ROI) that was calculated from the pre-migration references wells. The values shown represent the counted nuclei in the reference wells and after 18 hr of migration. Data shown are the means for n=24 wells per group +/- SD. C | Segmentation overlay showing cells inside and outside of the Detection Zone.

Key accomplishments

- migration assay ready for screening (easy to automate, robust readout)
- physiologically relevant readout
  - 3D information by extracellular matrix overlay
- cell by cell analysis of migrating cells
  - number and shape
- object-based quantifications:
  - morphological readouts on a single cell basis

3 Cell invasion / metastasis assay

The wells of an Oris™ Cell Invasion Assay plate were coated with a basement membrane extract extracellular matrix (ECM) and then populated with silicone stoppers. HT-1080 cells (3.5E4 / well) were plated at confluence and allowed to settle for 2 hr (37 °C, 5% CO2). After removal of the stoppers, each well was overlaid with ECM (+ 10 % FCS) to create a 3D environment. Cytochalasin D, which blocks actin polymerization, was used as benchmark compound for generating a dose-dependent inhibition of cell migration. Following a 48 hr incubation period (37 °C, 5% CO2) the cells were labeled with DAPI and Rhodamine-phalloidin (Invitrogen®).

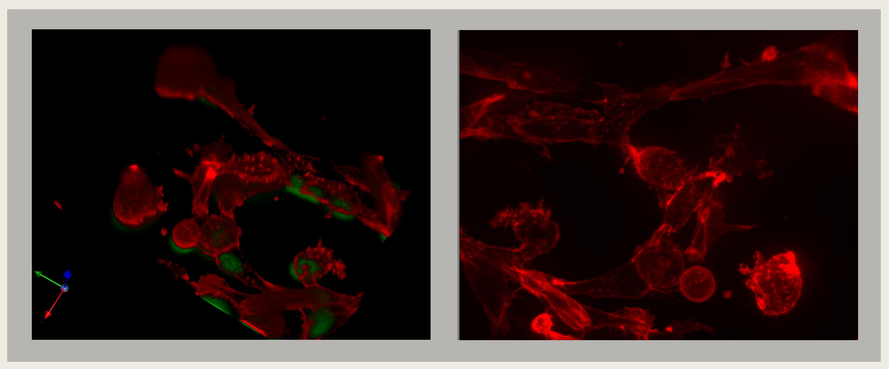

Figure 3. 3D representation (left) and maximum projection (right) of cells that invaded the ECM. Images were acquired using the Opera equipped with 60x water immersion objective as a stack of 31 layers with distance of 1 µm. Data representation was generated in Volocity®. Left | Cell protrusions (invadopodia) near the Detection Zone. These structures are associated with with proteolytic degradation of the ECM. False color overlay (red: actin staining, green: nuclei). Right | 2D representation of volumetric data as maximum projection.

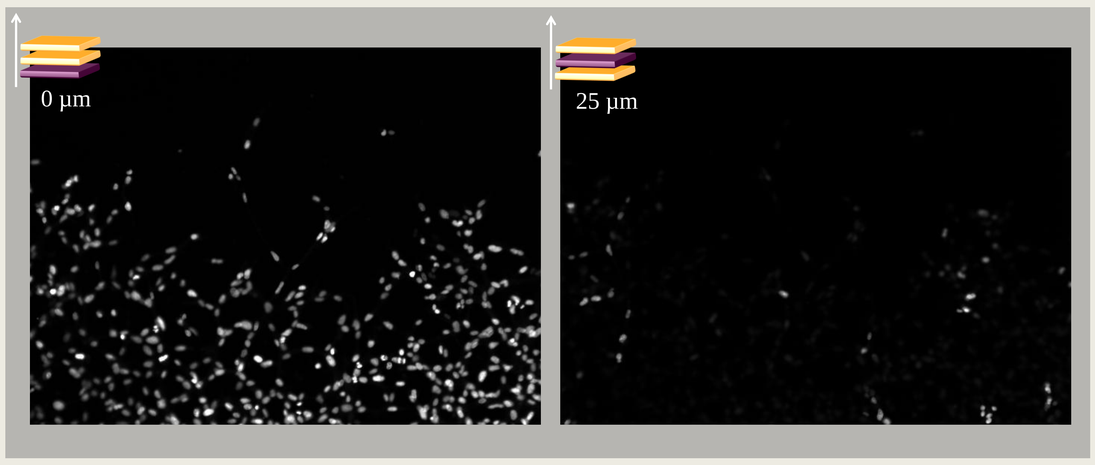

Figure 4. Nuclei in different layers of the matrix. Images were taken on the Opera using the 10x air objective after 48 hours of invasion. Left | Cells on plate bottom near the Detection Zone border. Right | Some cells could invade verticallyat least 25 µm , into the ECM.

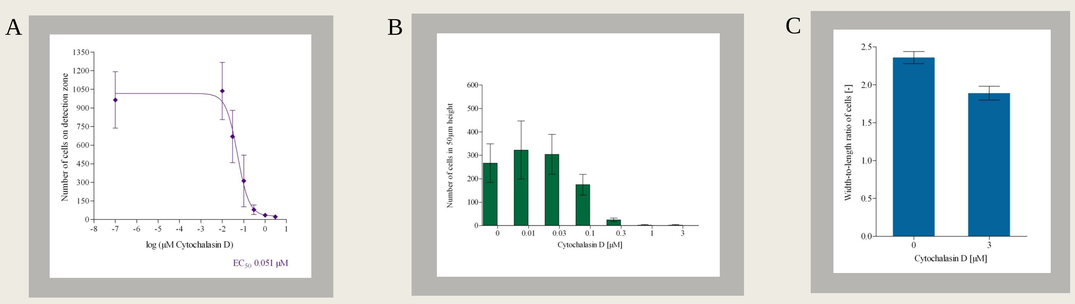

Figure 5. Data obtained by single-cell analysis of the invasion assay. A | Invasion in xy: In order to quantify invasion into the Detection Zone in the presence of the actin inhibitor Cytochalasin D the nuclei within this zone were counted. An EC_{50} value of 0.051 µM was calculated using the Prism software. B | Number of cells travelling from the confluent layer in z-direction. Cytochalasin D inhibits invasion in z-direction as it does in xy (A).
C | Comparison of width-to-length ratio of cells treated with and without Cytochalasin D. The cells treated with 3 µM Cytochalasin D exhibit smaller ratios compared to untreated cells indicating a rounder cell shape. Cytochalasin D inhibits the actin polymerization that affects the cytoskeleton arrangement. All data shown are the means for n=4 wells per group +/- SD.

4 Summary

Here, we present data acquisition and analysis from the Oris™ Cell Migration and Cell Invasion Assay using the Operetta and Opera HCS platform.
Using the Oris™ Assay platform on the Operetta and Opera resolves many limitations associated with the classical in vitro scratch assay [Yarrow *et al.*, 2004], such as high variability and cell disruption [Kam *et al.*, 2008].
The Oris™ Cell Invasion Assay enables quantification of cells penetraning into three dimensional extracellular matrix in a 96 well format. Confocal imaging and optical sectioning of the invasion layer allows robust and reliable counting of cells within the layers.

Kam Y, *et al.* (2008): A novel circular invasion assay mimics in vivo invasive behavior of cancer cell lines and distinguishes single-cell motility in vitro, BMC Cancer, 8, 198
Yarrow JC, *et al.* (2004): A high-throughput cell migration assay using scratch wound healing, a comparison of image-based readout methods, BMC Biotechnology, 4, 21

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