Development of a Wound Healing Assay in a High-Throughput Format using NYONE® Scientific

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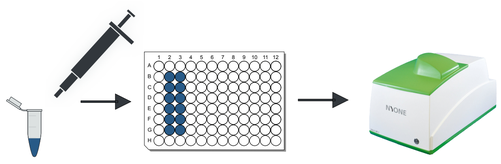
Background

Cell migration plays a crucial role in many physiological processes such as wound healing, tissue formation, and the immune response but it is also pathologically relevant in cancer invasion and metastasis. A convenient method to analyze cell migration is a wound-healing assay, in which an artificial cell-free gap (wound) is created on a confluent monolayer of cells. Closure of the wound is monitored over time by microscopy. However, with conventional or time-lapse microscopes, only a few samples can be measured at a time. Therefore, we aimed to develop a wound healing assay in a high throughput format using the automated microscope NYONE® Scientific and YT® software (SYNENTEC).

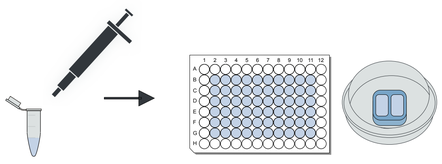
Experimental Setup

Count cells with Trypan Blue Application

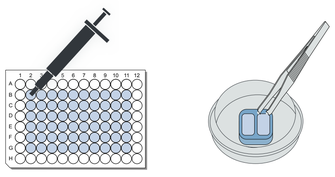


Optional: Stain cells with CellTracker™ Dyes

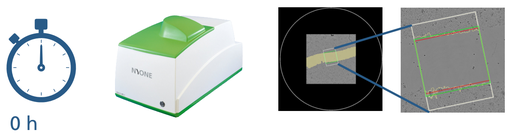
Seed cells



Create wounds

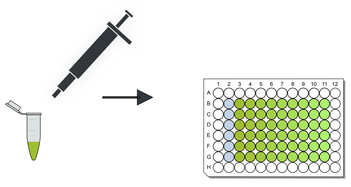


Measure in NYONE, phase 1 + 2



0 h

Treatment with Cytochalasin D



Measure in NYONE, phase 2

0 h
2 h
4 h
6 h
24 h

Results

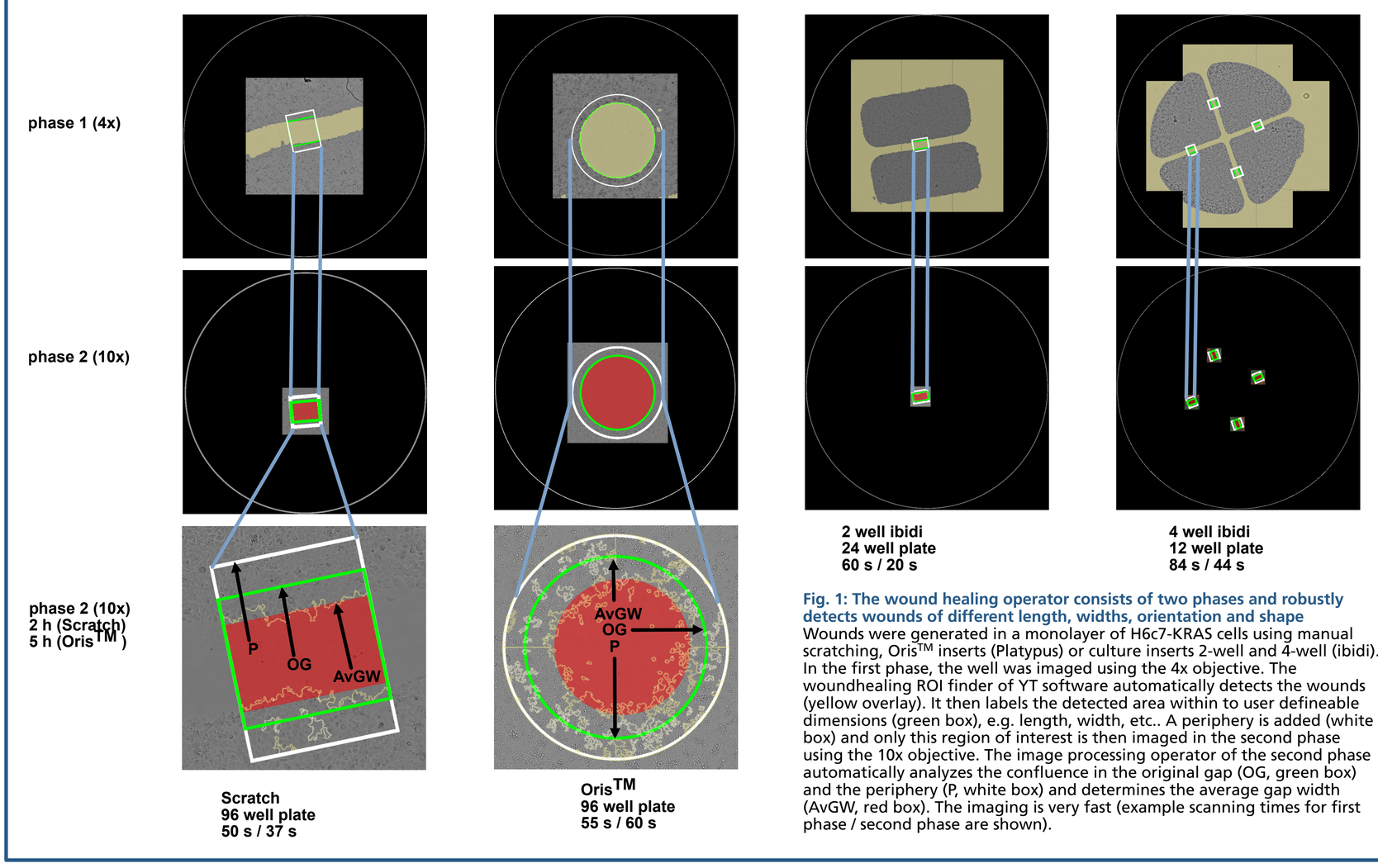


Fig. 1: The wound healing operator consists of two phases and robustly detects wounds of different length, widths, orientation and shape
Wounds were generated in a monolayer of H6c7-KRAS cells using manual scratching, Oris™ inserts (Platypus) or culture inserts 2-well and 4-well (ibidi). In the first phase, the well was imaged using the 4x objective. The woundhealing ROI finder of YT software automatically detects the wounds (yellow overlay). It then labels the detected area within to user defineable dimensions (green box), e.g. length, width, etc.. A periphery is added (white box) and only this region of interest is then imaged in the second phase using the 10x objective. The image processing operator of the second phase automatically analyzes the confluence in the original gap (OG, green box) and the periphery (P, white box) and determines the average gap width (AvGW, red box). The imaging is very fast (example scanning times for first phase / second phase are shown).

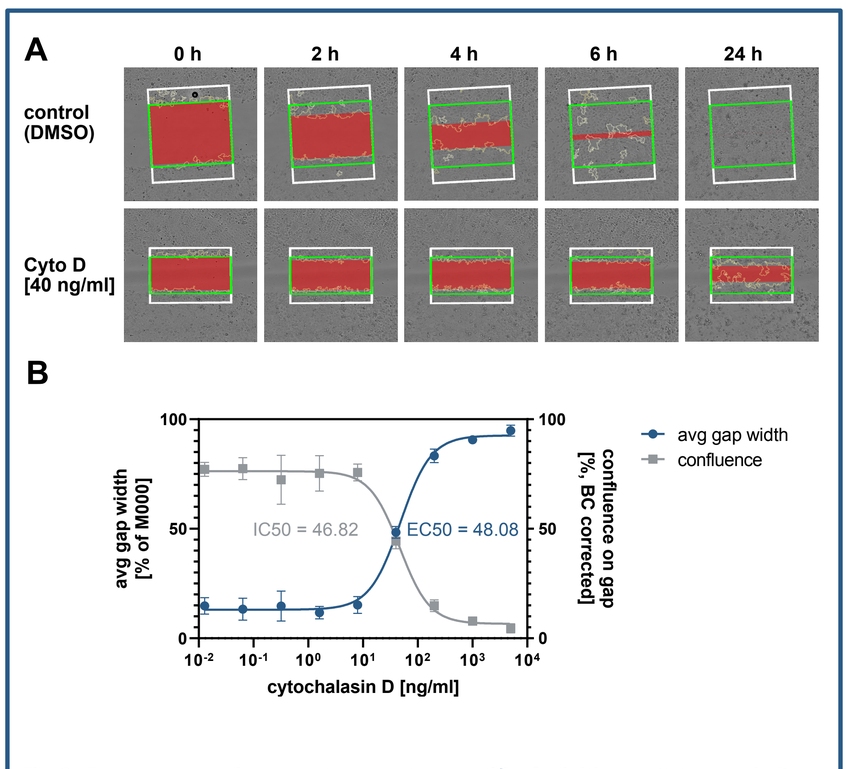


Fig. 2 : The wound healing operator robustly quantifies inhibition by Cytochalasin D
A confluent monolayer of H6c7-Kras cells in 96 well plates was manually scratched with a pipette tip and then treated with the inhibitor of actin polymerisation Cytochalasin D. The images were analyzed using YT® software and the generated data was fitted and plotted in GraphPad Prism.

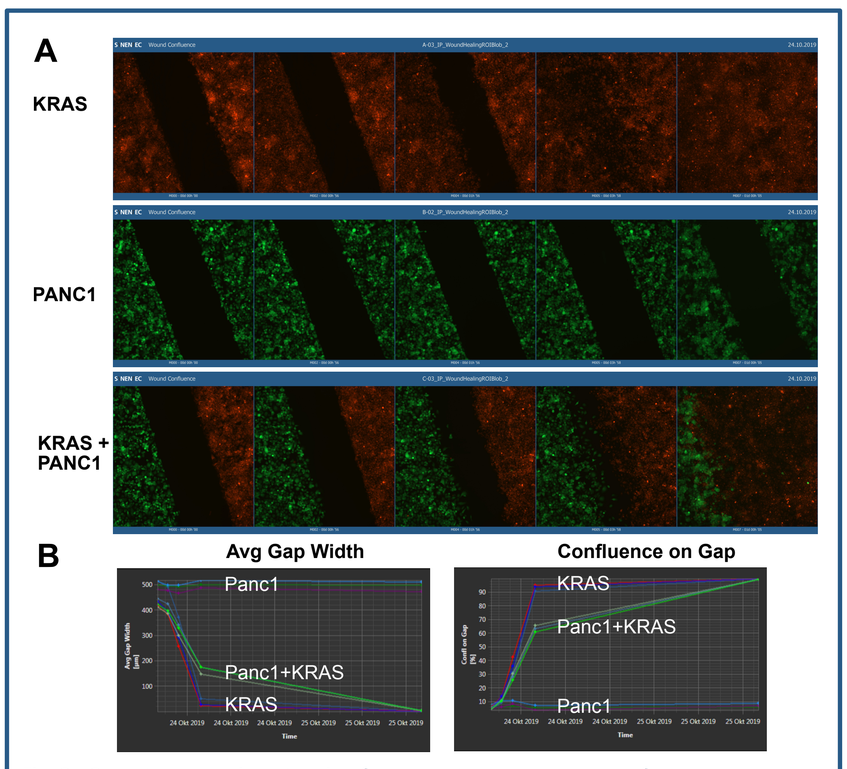


Fig. 3 : Co-culture experiments using fluorescently labelled cells are fastly measured and quantified by the operator
H6c7-Kras (red) and PANC1 cells (green) were stained with CellTrackerTM dyes and seeded in ibidi inserts. The images were automatically exported as galleries (A) and quantified (B).

Institut für Experimentelle Tumor-forschung

The newly developed wound healing operator

- **Detects wounds of different width, length, orientation and shape**
- **Is very fast**
- **Automatically analyzes the images**

is a great tool for high throughput screening.