

# Automated imaging and analysis of collective cell migration

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## Introduction

Collective cell migration is essential for normal development and functioning of the body. It is of importance for processes such as angiogenesis and wound repair. However, cell migration is also involved in a multitude of pathologies, including cancer metastasis.

In order to get more insight into collective cell migration, a variety of cell migration assays have been developed. One of the easiest and mostly used methods to investigate cell migration is the scratch assay (also called the wound healing assay). The scratch assay starts with the culture of a confluent monolayer of cells. Thereafter, a scratch is made in the monolayer to create a "wound", this is generally done with a pipette tip. An image of the scratch is taken with a microscope at the initial and later timepoints to analyze the closure of the scratch over time. Generally, the scratch width, the percentage of closure and/or the migration speed are parameters derived from this assay.

One of the downsides of the scratch assay is that only a small part of the scratch is analyzed due to the small field of view of the standard benchtop microscope. Other downsides are that it

is difficult to find the exact same spot after placing the samples back in the incubator and that the analysis is commonly done manually, making it a labor-intensive process. Next to this, the cells experience a temperature shock every time they are taken out of the incubator to take images of the scratch.

Automated live-cell imaging and analysis could provide a solution to overcome these issues. The CytoSMART® Omni is an automated live-cell imager that can be placed inside a regular cell-culture incubator. This device is capable of imaging an entire well plate at regular intervals, eliminating the temperature shock and the difficulty to find the same part of the scratch at each timepoint. Since the CytoSMART® Omni images the entire well plate, the complete scratch area of all wells of the well plate can be analyzed simultaneously.

This study provides a proof-of-principle of scratch assay imaging and analysis using the CytoSMART® Omni and its cloud-based scratch analysis algorithm. C6 (rat glioma) cells were treated with different concentrations of Paclitaxel to investigate the effect of Paclitaxel on collective tumor cell migration.

## Material and methods

A 24-well plate was seeded with C6 cells at a density of 50,000 cells/cm<sup>2</sup> in Advanced DMEM (Invitrogen) supplemented with 10% FBS (Gibco) and 1% penicillin-streptomycin (Gibco). After 24 h of culture (37°C and 5% CO<sub>2</sub>), a scratch was made in the cell monolayer using a plastic 200 µl pipette tip. The samples were rinsed with PBS (Gibco) to remove the detached cells, thereafter, medium supplemented with 0, 1, 10 or 100 nM of Paclitaxel (PX; Invitrogen) was added to the cells.

The well plate was subsequently placed on the CytoSMART® Omni (37°C and 5% CO<sub>2</sub>; Fig. 1) to make a high-resolution image every hour for 23 h. These images were uploaded to the CytoSMART® Cloud to determine the scratch area and average migration speed of each individual well using the CytoSMART® scratch analysis algorithm.

The data was downloaded from the CytoSMART® Cloud and the percentage of scratch closure was calculated to normalize the scratch area. Differences in scratch closure were analyzed

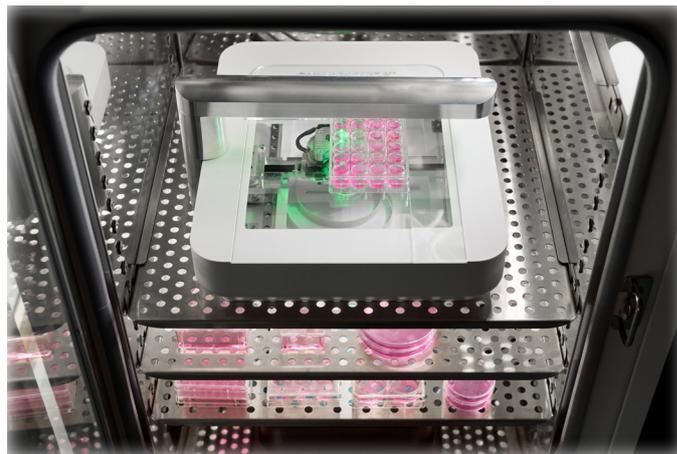


Figure 1. The 24-well plate placed on the CytoSMART® Omni that is placed inside a cell culture incubator.

with SPSS Statistics (IBM) using one-way ANOVA followed by a Bonferroni post-hoc test and were considered significant at  $p < 0.05$ .

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## Results

Immediately after making the scratch, cells start to migrate in all four groups treated with different concentrations of PX (Fig. 2). The scratch area of the 0 and 1 nM PX treated groups is (almost)  $0 \mu\text{m}^2$  after 23 h, while the scratch area of the 10 and 100 nM PX treated groups is  $1.3 \cdot 10^6 \mu\text{m}^2$  and  $4.5 \cdot 10^6 \mu\text{m}^2$ , respectively. Two hours after the addition of PX, the percentage of scratch closure of the 10 nM and 100 nM PX samples is significantly lower compared to the control samples (Fig. 3). One hour later, the scratch closure of these two groups is significantly lower compared to the 1 nM PX group as well. Starting from 5 h, the

scratch closure of the 100 nM PX samples is also significantly lower than that of the 10 nM PX samples.

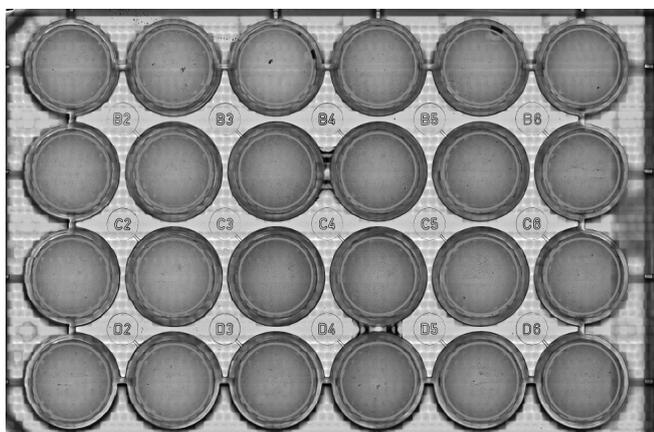
The average migration speed of the samples treated with 100 nM of PX ( $1.7 \cdot 10^5 \pm 0.7 \cdot 10^5 \mu\text{m}^2/\text{h}$ ) is more than two times lower compared to the other three groups (Fig. 4). The average migration speed of the 10 nM PX group ( $3.3 \cdot 10^5 \pm 0.7 \cdot 10^5 \mu\text{m}^2/\text{h}$ ) is significantly lower compared to the untreated samples ( $4.1 \cdot 10^7 \pm 2.2 \cdot 10^5 \mu\text{m}^2/\text{h}$ ) and the samples treated with 1 nM of PX ( $3.9 \cdot 10^7 \pm 1.7 \cdot 10^5 \mu\text{m}^2/\text{h}$ ).

## Discussion

Since scratch assays are easy to perform and low costs, they are widely used to study collective cell migration. However, it is difficult to analyze the exact same part of the scratch over time if the samples are placed back in the incubator between timepoints. Together with the small part of the scratch that is analyzed and the manual analysis, it can make the scratch assay analysis cumbersome and less accurate. Next to this, the cells experience a temperature shock every time they are taken out of the incubator to take images of the scratch. The problems can be overcome by using the CytoSMART® Omni, a live-cell imager that can be used inside a cell-culture incubator. In this study, we provide a proof-of-principle of scratch assay imaging and analysis using the CytoSMART® Omni.

We have shown that collective cell migration of C6 cells is significantly decreased upon treatment with 10 or 100 nM of PX. This impaired collective cell migration is shown by the decreased percentage of scratch closure over time and the decreased average migration speed. PX inhibits collective cell migration by stabilizing microtubules. Normally, dynamic microtubules push the lamellipodia forward and help regulate actin polymerization and focal adhesion formation at the leading edge of the cell [1, 2]. On the trailing end of the cell, disassembly of the microtubules leads to increased actin contractility [1, 2]. Next to this, the microtubules are involved in focal adhesion disassembly at the trailing end. Together, this leads to movement of the cell in the direction of the leading edge. In case the microtubules are stabilized by PX, the above-

### whole well view



### follow scratch area reduction over time

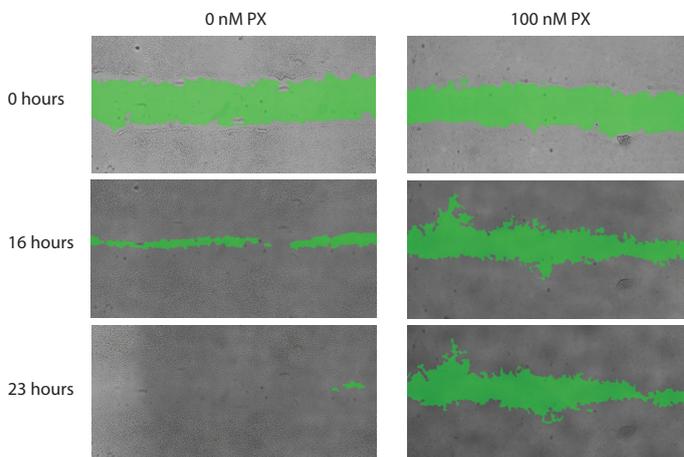


Figure 2. (A) A high-resolution scan of the 24-well plate taken at 0h. (B) Zoomed in images of the blue boxes in A. For each of the groups, the scratch detected by the Scratch analysis algorithm is false colored in blue at the first timepoint (0 h) and green at the later timepoints (8, 16 and 23 h).

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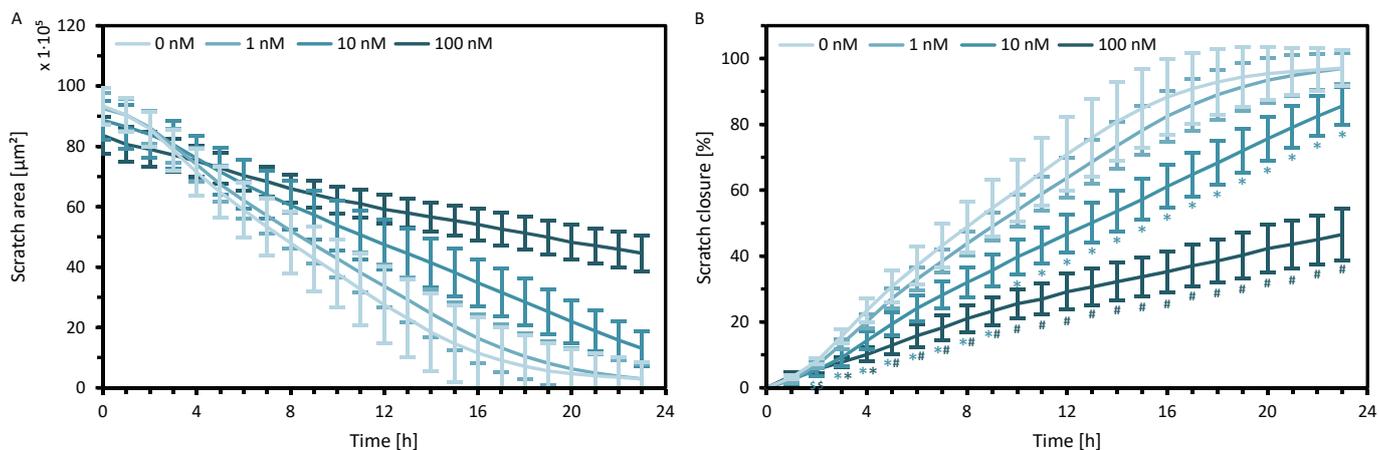


Figure 3. (A) Decrease in scratch area over time for the samples treated with four different concentrations of PX (mean  $\pm$  standard deviation; n=6). (B) Increase in the percentage of scratch closure over time for the samples treated with four different concentrations of PX (mean  $\pm$  standard deviation; n=6). \$: Significantly lower than 0 nM PX, \*: significantly lower than 0 and 1 nM PX, #: significantly lower than 0, 1 and 10 nM (p<0.05). The color of the sign represents the color of the group.

mentioned processes are inhibited, which in turn inhibits cell migration.

Liao et al. have shown that upon addition of 10, 50 and 100 nM of PX, the migration rate of NRK fibroblast is inhibited after 2 hours of treatment [3]. This corresponds to the significantly decreased scratch closure of the 10 and 100 nM treated samples observed after 2 hours of treatment, in this study. Like PX, the microtubule stabilizing drugs Docetaxel and Zampanolide, inhibit cell migration [4]. A recent study by Field and colleagues has shown that scratch closure is reduced by more than 40% after 18 h of treatment with different concentrations (2 – 15 nM) of Docetaxel or Zampanolide [4].

Since the CytoSMART® Omni is placed inside a cell-culture incubator to image cell migration every hour, the cells do not have to be taken out of the incubator. This eliminates the temperature shock that the cells experience when taking images with a standard benchtop microscope. Moreover, since the well plate is not translocated during the experiment, the exact same spot can be monitored over time, increasing the accuracy of the data. The data accuracy is also increased by analysis of the entire scratch instead of analysis of only a (few) part(s) of the scratch. Lastly, since all wells of the well plate are imaged and analyzed

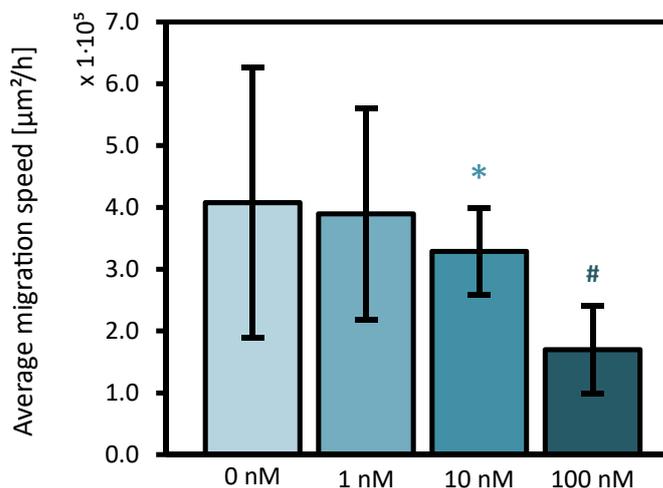


Figure 4. The average migration speed of the samples treated with four different concentrations of PX (mean  $\pm$  standard deviation; n=6). \*: Significantly lower average migration speed than 0 and 1 nM PX, #: significantly lower average migration speed than 0, 1 and 10 nM of PX (p<0.01).

automatically, the cumbersome and labour-intensive manual imaging and analysis process is eliminated, which makes this new method of scratch assay imaging and analysis very time-efficient.

## Conclusion

In this study, we have shown that the CytoSMART® Omni and its automated, cloud-based, CytoSMART® scratch analysis algorithm can be used to easily image and analyze scratch assays. The CytoSMART® Omni made a high-resolution scan every hour for 23 h and the CytoSMART® scratch analysis algorithm accurately determined the scratch area of each well at all timepoints. Using this method, we have shown that PX

inhibits collective cell migration of C6 cells in a dose dependent manner, starting from 10 nM of PX. Since all 24 wells of the 24-well plate were automatically imaged and analyzed at each timepoint, the throughput, time-efficiency and accuracy of the scratch assay is increased. Furthermore, the CytoSMART® Omni also provided the benefit of monitoring the scratch closure at optimal culture conditions.

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## References

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