Application Note

IPRASENSE
CELL CULTURE MONITORING INSTRUMENTS

Keywords or phrases

Cell migration, scratch assay, real-time imaging, wound healing, disease progression, in vitro experiments

Automated Continuous Monitoring Of Cell Migration - Scratch Assay

Abstract

Cell migration is essential for a variety of biological processes, including development, wound healing and disease progression. The Scratch Assay is a common method for studying cell migration in vitro. This note highlights the use of **CYTONOTE** in scratch assays, offering real-time imaging and precise monitoring. **CYTONOTE** allows timely intervention and distance measurement using **HORUS** software, enhancing the study of cell migration, including tumor cell invasion.

Introduction

Cell migration is a central process in the development and maintenance of multicellular organisms. Tissue formation during embryonic development, wound healing and immune responses all require the orchestrated movement of cells in particular directions to specific locations. Cells often migrate in response to specific external signals, including chemical and mechanical signals. Errors during this process have serious consequences, including intellectual disability, vascular disease, tumor formation and metastasis. An understanding of the mechanism by which cells migrate may lead to the development of novel therapeutic strategies for controlling, for example, invasive tumor cells. Cell migration and invasion play a role in many

normal and pathological processes including immune responses, embryonic development, angiogenesis, regeneration, tumor metastasis and wound healing.

In in vitro experiments, Scratch assay is the primary method for assessing cell migration and cell invasion. In this application note, we will describe this test through measurements with the **CYTONOTE**, through the study of two publications, one from the Namur Research Institute for Life Sciences (NARILIS) with Clarins and one from the Institute of Genetic Medicine of Newcastle University with ReGenesys.

We will see in the following sections, how the **CYTONOTE** was used for the scratch assay.

Material & Methods

1. The CYTONOTE and HORUS Software

The **CYTONOTE** is able to perform measurements inside the incubator and to recognize cells without any labelling. The **HORUS** software calculates many parameters automatically such as cell number, cell saturation, cell area, cell morphology. Unlike a conventional microscope, the absence of focus in the **CYTONOTE** allows an extremely wide field of view.

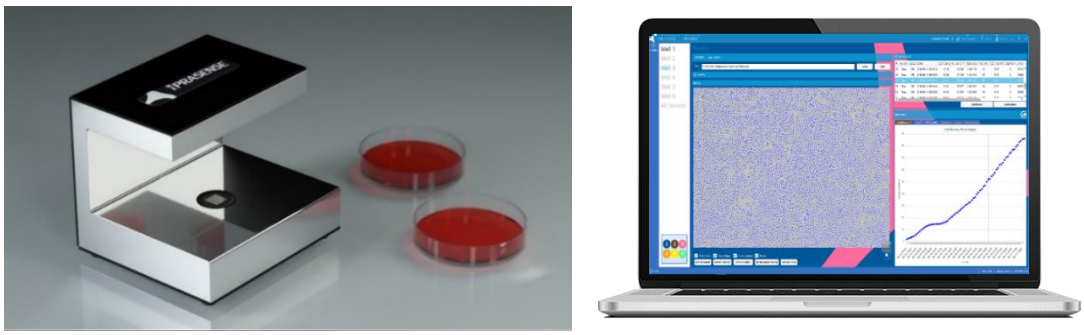

*Figure 1 : **CYTONOTE** 1W on the left and **HORUS** Software on the right*

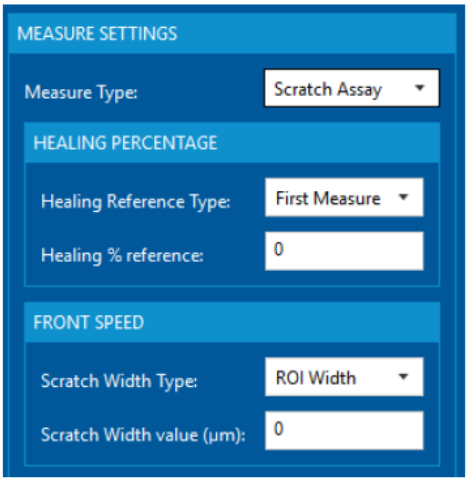

Figure 2 : *Measure settings in Scratch Assay by* ***HORUS*** *Software*

2. Cell Migration Assay

The **CYTONOTE** provides complete images and data. Scratch assay assesses cell migration and cell invasion (A). Scratch assay quantifies cell migration by a carpet of cells (so 100% confluent), make a scar in the middle of the carpet with pipetting (B) and see how long it closes. This method is used in particular for tumor cells, for research on cancers and cellular invasions. The **CYTONOTE** records the healing of scratches in real time and also calculates the percentage of closure of scratches (C) and the migration rate of cells (D) for example.

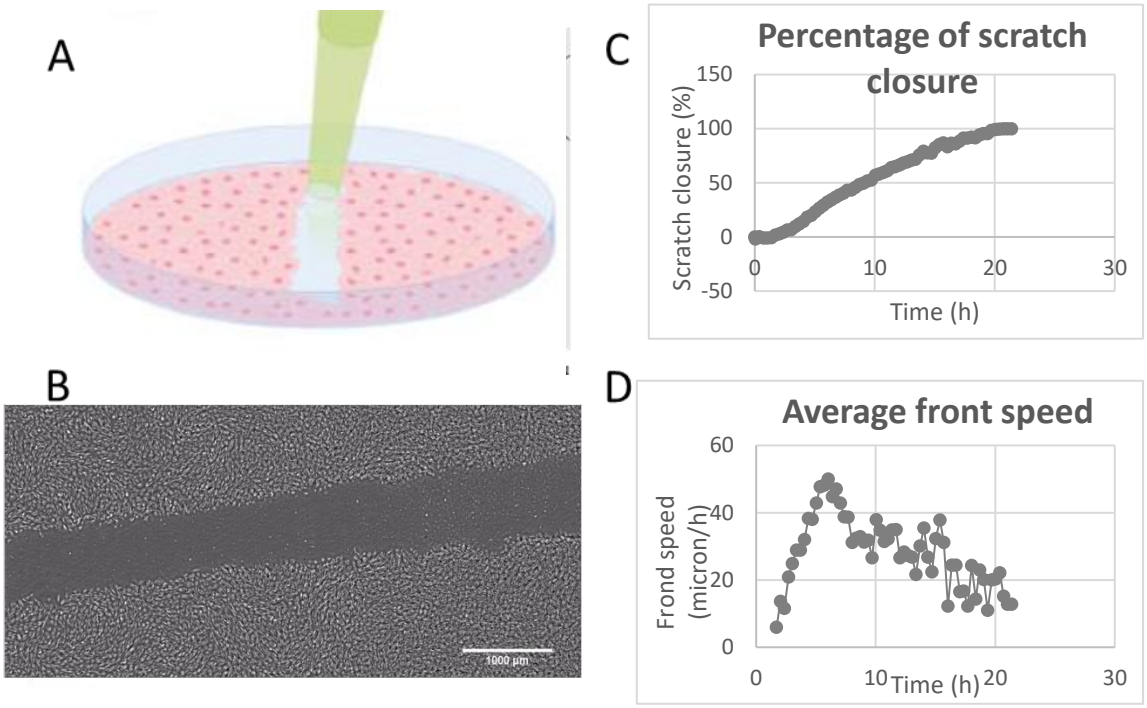

Figure 3: *Scratch assay*

(A) The monolayer was scraped with a p200 or a 1 mL micropipette tip (dipending on articles) in a straight line to create a cell-free "scratch"
(B) Scratch scar of human fibroblast cells in Petri dish (video is available on Youtube "Wound healing" https://www.youtube.com/watch?v=F4-YFK0gHZs;
(C) Real-time quantitative scratch closure
(D) Real-time quantitative scratch cell edge migration rate (micro / h)

3. Cells

Different cell lines can be observed with the **CYTONOTE**. In this application note, the following cell lines were used:

- Human epidermal keratinocytes (Mound A. et al.),
- Human corneal stromal cells (Al-Jaibaji O. et al.).

The cells were grown in these experiments in Petri dishes (Mound A. et al.) but also in culture flasks (Al-Jaibaji O. et al.), in an incubator at 37°C.

The monolayer can be scraped in a straight line to create a cell-free "scratch" as was done in the paper by Mound A. et al. The cells can then be treated with different active ingredients to observe the effect on cell migration.

Results

Examples of results obtained with the **CYTONOTE** are discussed in this section.

In the publication by Mound A. et al, the **CYTONOTE** allowed to observe (**Figure 4**):

- The abolition of keratinocyte migration caused by cholesterol depletion by MβCD (7.5 mM);
- The slowing down of the process caused by the degradation of sphingomyelin in the plasma membrane of keratinocytes by incubation of the cells with SMase (5 mU/ml);
- Cell migration of Y27632.

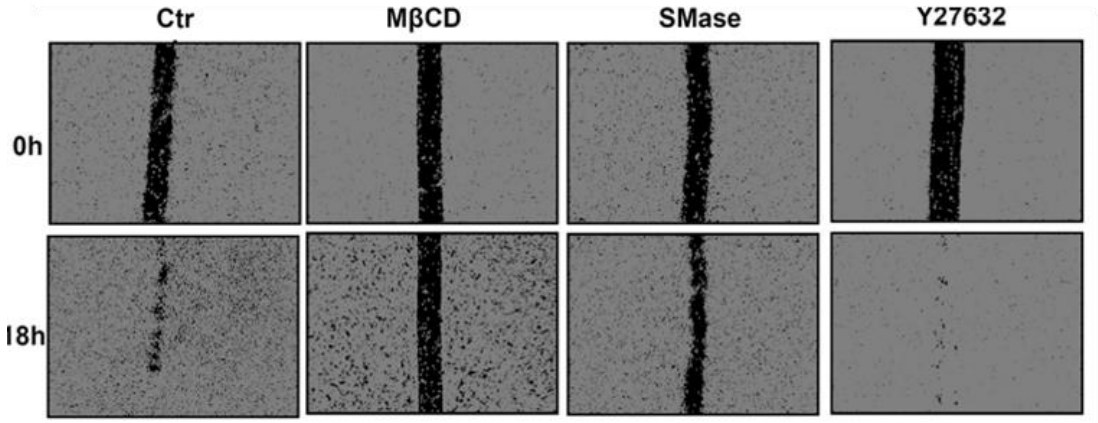

***Figure 4** : Images of Scratch assay with 4 conditions*

Same size scratches of same keratinocytes were treated with different medications (MβCD: methyl-β-cyclodextrin treatment for cholesterol depletion, SMase: Bacillus cereus sphingomyelinase treatment for sphingomyelin depletion, Y27632: compound for treatment with Rho kinase inhibitor) at 0h. And the scratches were closed at 18 h.

In the publication by Al-Jaibaji O. et al. the **CYTONOTE** allowed to observe (**Figure 5 and 6**):

- The migration of human corneal stromal cells to the cell-free zone.
- The improvement of wound area closure over time in the presence of encapsulated MAPC compared to controls.
- The tracking of the scratch area in mm^2 over time.
- The percentage of scratch closure obtained automatically via the integrated software.

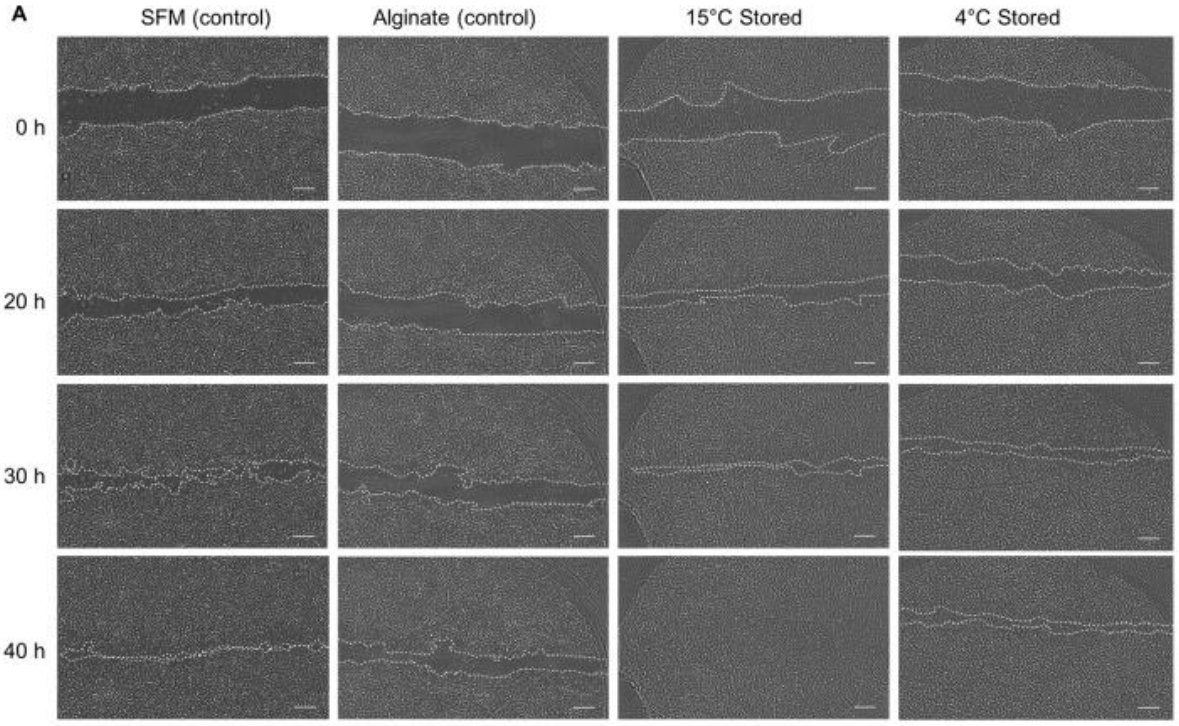

Figure 5 : *Images of Scratch assay with 4 conditions*

Representative images using time-lapse microscopy of the scratch-wound of corneal stromal cells treated with serum-free medium (SFM), alginate, or 1 x 106 MAPC alginate + MAPC at time points 0, 20, 30 and 40 hours.

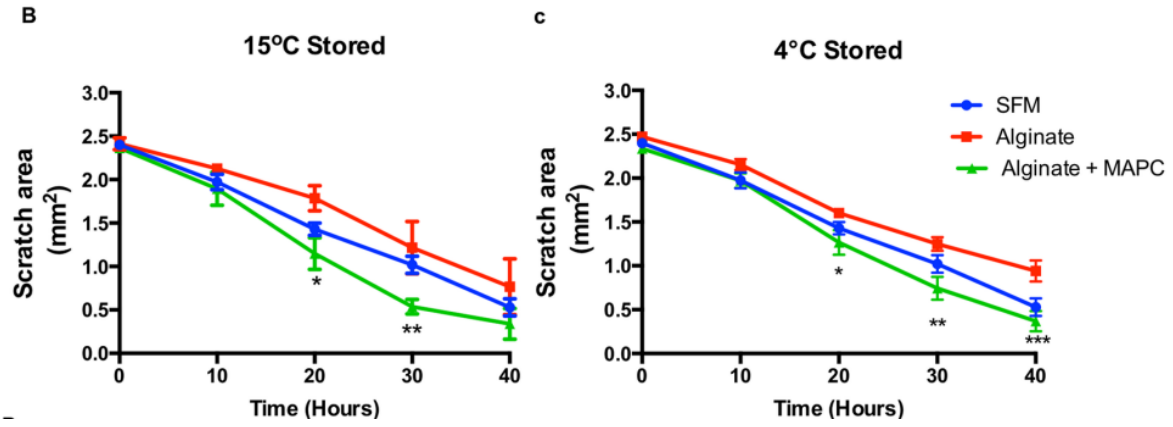

Figure 6 : *Scratch area (mm²) as a function of time. Treatments were stored for 72 hours at 15˚C (B) or 4˚C (C)*

Conclusion

The **CYTONOTE** allows to analyse cell migration ability (such as tumor cell invasion ability for example) using time-lapse imaging. Real-time monitoring provides constant quality control for the cells allowing the user to add a compound at good timing for example. The distance of cell migration is measured by the **HORUS** software in real-time recording.

References

(1) Online version available at https://www.iprasense.com/live-cell-imaging-automatic-cell-counters-applications/migration-assay

(2) Videos: Migration assay - IPRASENSE
Angiogenesis - IPRASENSE
Time-lapse images of cell - YouTube

(3) Mound, A., Lozanova, V., Warnon, C., Hermant, M., Robic, J., Guere, C., ... & Poumay, Y. (2017). Non-senescent keratinocytes organize in plasma membrane submicrometric lipid domains enriched in sphingomyelin and involved in re-epithelialization. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1862(9), 958-971. https://www.sciencedirect.com/science/article/pii/S138819811730104X?via%3Dihub

(4) Al-Jaibaji, O., Swioklo, S., Gijbels, K., Vaes, B., Figueiredo, F. C., & Connon, C. J. (2018). Alginate encapsulated multipotent adult progenitor cells promote corneal stromal cell activation via release of soluble factors. Plos one, 13(9), e0202118. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0202118