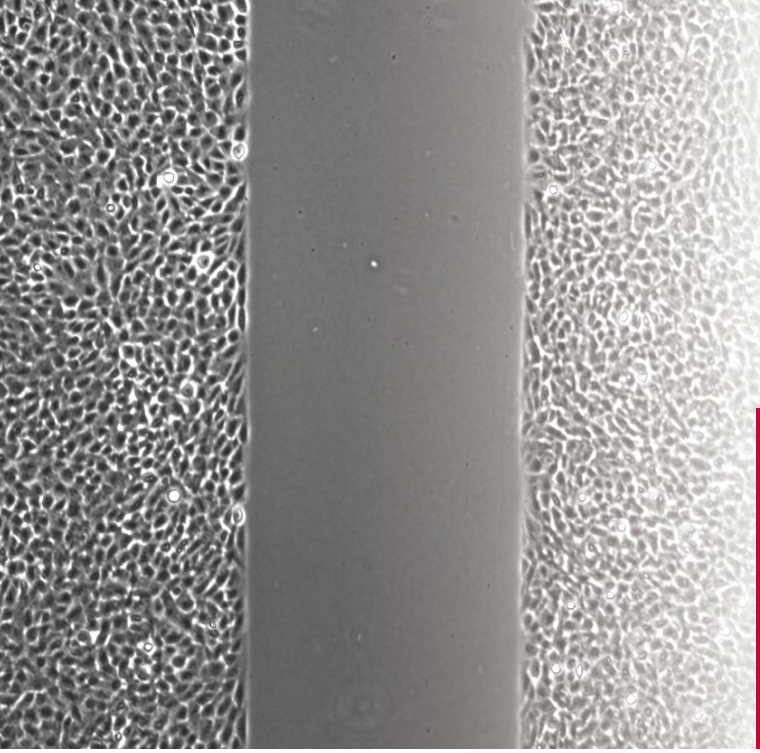


cells in focus

ibidi Application Guide

Wound Healing and Migration Assays

Selected Publications

M. Caesar, S. Zach, C. B. Carlson, K. Brockmann, T. Gasser and F. Gillardon. Leucine-rich repeat kinase 2 functionally interacts with microtubules and kinase-dependently modulates cell migration. Neurobiology of Disease, 2013, 10.1016/j.nbd.2012.12.019,

Y. Shih, M. Wang, H. Peng, T. Chen, J. Chang and J. Chiu. Modulation of Chemotactic and Pro-Inflammatory Activities of Endothelial Progenitor Cells by Hepatocellular Carcinoma. Cellular Signalling, 2012, 10.1016/j.cellsig.2011.11.013

T. Yan et al. Hypoxia Regulates mTORC1-Mediated Keratinocyte Motility and Migration via the AMPK Pathway. PLOS ONE, 2017, 10.1371/journal.pone.0169155

The Principle of Wound Healing and Migration Assays

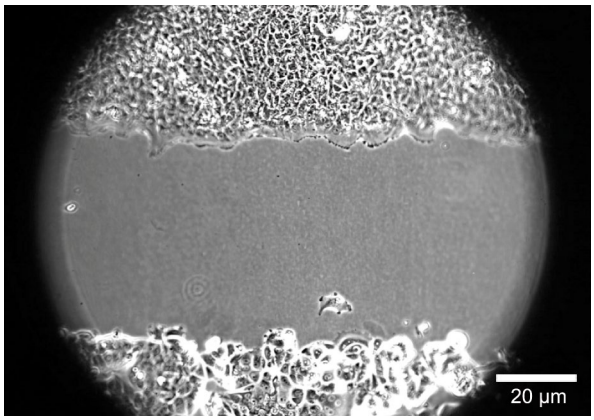
Applications of Wound Healing and Migration Assays

Cell migration occurs during many important physiological processes. For example, controlled cell migration allows for embryonic development, tissue injury, and wound healing. In contrast, cell migration is dysregulated in many pathological situations, such as cancer metastasis and inflammation. Not surprisingly, the principles behind cell migration have been studied in many contexts.

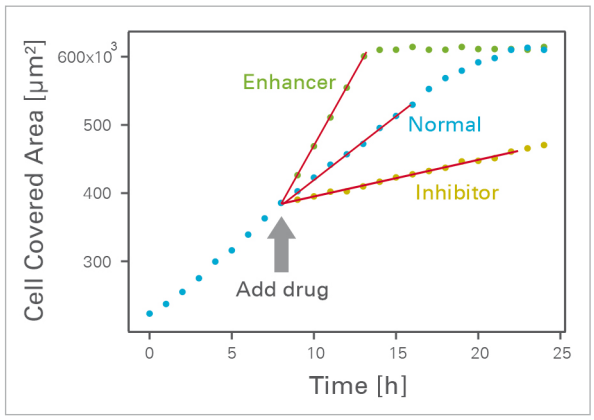
Wound healing and migration assays are widely used approaches for the analysis of cell migration under different conditions. They give insight into the following relevant scientific topics:

- **Conditional Migration and Wound Healing Studies:** How do the cells migrate under different conditions (e.g., after being treated with a specific compound/enhancer/inhibitor, after gene silencing using siRNA or CRISPR/Cas9, after changing substrate stiffness, or after changing matrix composition)?
- **High Throughput Drug Screening:** Which drug alters the migration rate of a specific cell type (e.g., cancer cells)?
- **Cell Interaction Studies:** Do the cells remain in contact or do they migrate singularly? How do the cells interact with each other during migration? What is the cell-autonomous migration ability?
- **2D Invasion Assays:** How do two different cell types interact with each other (e.g., tumor cells and fibroblasts)?

In contrast to general wound healing and migration assays, directed migration assays, such as chemotaxis assays, measure a gradient-dependent cell movement in a 2D or 3D environment.



Two different cell types in a 2D invasion assay. Data provided by C. Matern and K. D. Nnetu, University Leipzig, Germany.



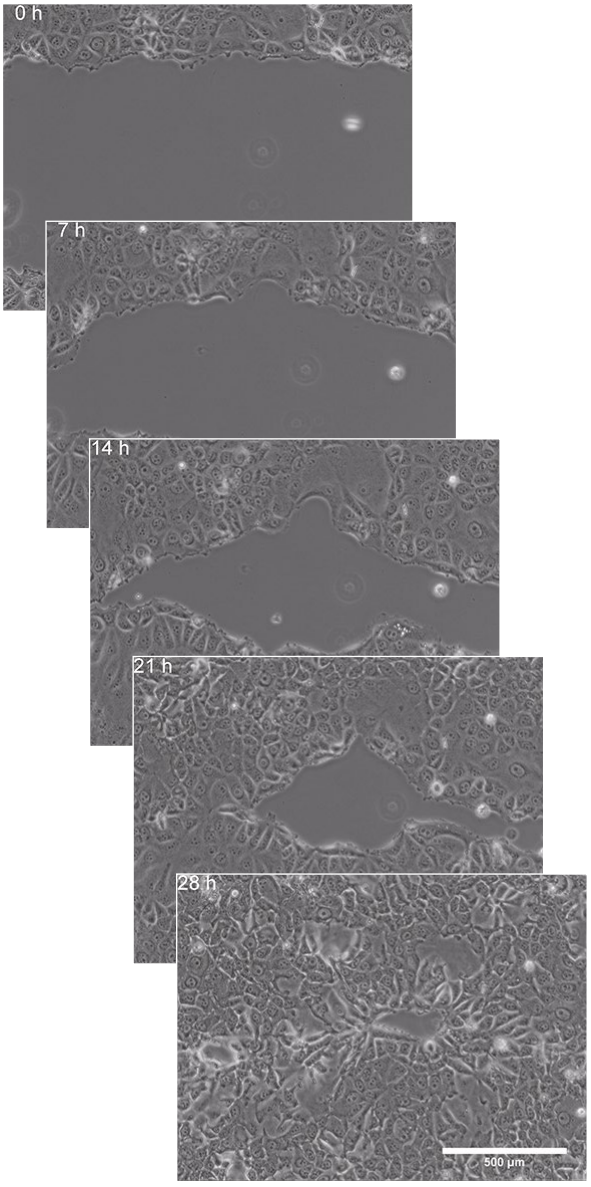
Example analysis of the influence of inhibitors or enhancers on wound healing.

How to Perform a Wound Healing and Migration Assay

The most characteristic readout of a wound healing and migration assay is the change of the cell-covered area (gap closure) over time. Conducting a wound healing and migration assay is an easy procedure:

- Create a physical gap within a cell monolayer.
- Monitor the process of cell migration into the gap with live cell imaging or by taking photos at different time points.
- Analyze the gap closure rate, which is a typical experimental readout, manually or by using automated software.

Despite the apparent simplicity of a wound healing assay, many factors can influence the experimental outcome and they have to be controlled. In the following chapters, you will learn about the principle and the setup of a wound healing assay. In addition, we will discuss the parameters that need to be standardized in order to achieve reproducible, robust results.

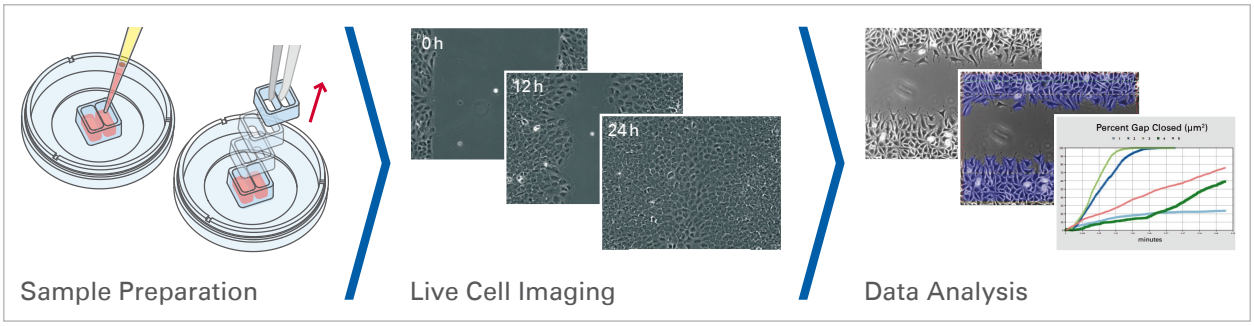


Live cell imaging shows the gap closure of MCF7 cells after wounding.

J.E.N. Jonkman, et al. An introduction to the wound healing assay using live-cell microscopy. Cell Adh Migr, 2014, 10.4161/cam.36224

W.J. Ashby, A. Zijlstra. Established and novel methods of interrogating two-dimensional cell migration. Integr Biol 2012, 10.1039/c2ib20154b

Experimental Workflow



Before Starting

The following questions should be addressed before starting the experiment:

Is it possible to reproduce cell culture conditions?

Since many external factors can influence the outcome of a wound healing assay, a standard approach should be developed in advance. All procedures need to be kept consistent throughout the experiment. If possible, the same devices, cell passages, number of medium changes, reagents, and disposables should be used for every experiment.

Which cell culture vessel should be used?

Cell culture vessels need to have a thin bottom (~170 µm) that is optimized for high-resolution live cell imaging and inverted microscopy (e.g., the ibidi Polymer Coverslip). The vessel size should be sufficient for convenient gap creation. Depending on the number of samples, either the ibidi Culture-Inserts in the µ-Dish 35 mm, high or the µ-Plate 24 Well can be used. In case there is no need for high-resolution fluorescence microscopy, the Culture-Inserts can be self-inserted into a large variety of cell culture formats, such as petri dishes, cell culture chambers, and multiwell plates.

Which substrate should be used?

The substrate depends on the cell type and the experimental conditions. A tissue-treated substrate that provides sufficient cell attachment, like the ibidi Polymer Coverslip, works for most cell types. Some cell types, however, require a special coating. In the ibidi Application Note Cell Culture Coating (PDF), you will find detailed information on how to do your own coating on µ-Slides and µ-Dishes.

Is it necessary to inhibit cell proliferation?

During a wound healing assay, cells not only migrate into the gap, but they also proliferate. This can be suppressed by treating the cells with a proliferation inhibitor, such as mitomycin or actinomycin C, before creating the gap. The dosage and the appropriate controls must be determined in advance so that the cells do not undergo apoptosis or alter their migration capability due to the cytostatic drug.

Which cell density is ideal?

Ideally, the assay should be carried out directly after an optically confluent cell monolayer has formed. The cell seeding density needs to be defined separately for each cell type. Optimally, the cells should form a monolayer 24 hours after seeding.

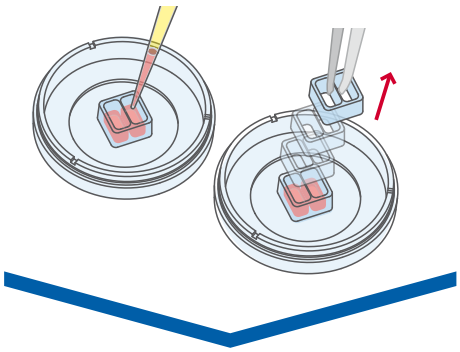
How is the gap created?

The consistency of gap creation is highly important when conducting a wound healing assay. Several methods for creating a gap in a cell monolayer have been developed: scratching with a needle or pipet tip, placing an insert or stencil, chemical surface treatment, or the application of electricity. Find more information about the advantages and drawbacks of different gap creation methods here.

How is the gap closure monitored?

It is possible to observe the gap closure by taking pictures at different time points. However, for most experimental setups, live cell imaging is the most accurate and convenient way to monitor the gap closure.

Sample Preparation



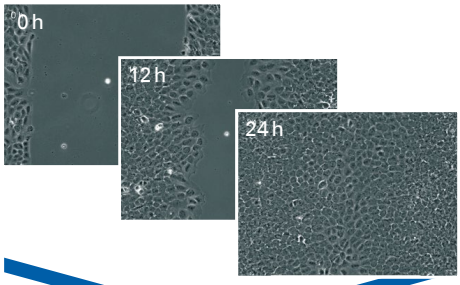
Procedure:

Here, the gap is created by using the ibidi Culture-Inserts, which guarantee extremely high reproducibility due to the defined size of the cell-free gap. Choose a suitable Culture-Insert in an appropriate cell culture vessel. Seed and culture the cells until they form an optically confluent monolayer. Let the cells grow for approximately 24 hours. If necessary, treat the cells with a proliferation inhibitor, then remove the Culture-Insert to create the gap.

ibidi Solutions:

- The ibidi Culture-Insert 2 Well | 3 Well | 4 Well are silicone inserts with a defined, cell-free gap for wound healing, migration, 2D invasion assays, and co-cultivation of cells. They are available as individual inserts in a µ- Dish$^{35\ mm}$ or as 25 pieces in a transport dish for self-insertion, and provide a quick and easy experimental setup.
- The Culture-Insert 2 Well 24 is a ready-to-use solution in a 24 well plate for cost-effective high throughput assays.
- The ibidi µ-Slide 2 Well contains 2 wells that have the ideal size for the insertion of Culture-Inserts.

Live Cell Imaging



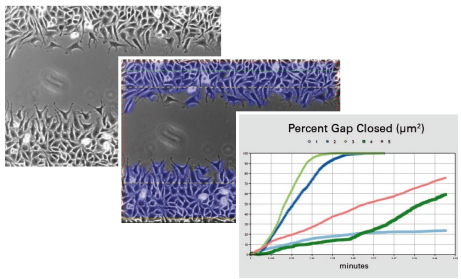
Procedure:

The cells should be monitored immediately after the gap creation, in most cases by using phase contrast microscopy. It is possible to take pictures of the gap at different time points (e.g., after 6 and 12 hours) or do live cell imaging. Usually, it is sufficient to monitor the cells for 24 hours or less. For an accurate analysis, it is important to observe the exact same gap position during the entire experiment.

ibidi Solutions:

The ibidi Heating and Gas Incubation System provides a physiological environment under the microscope, enabling live cell imaging during wound healing and migration assays.

Data Analysis



Procedure:

A typical wound healing and migration assay generates gigabytes of imaging data that have to be further processed and analyzed by manual image analysis or by using automated software. In many experimental setups, the main readout is the speed of the wound closure. Other possible readouts are the measurements of the cell-free area and the cell-covered area.

ibidi Solutions:

With the Wound Healing ACAS Image Analysis software, ibidi provides a time-saving, fully automated solution for the quantification of wound healing and migration assays. It includes analyses of the cell-free area, the cell-covered area, and the speed of wound closure.

Creating the Gap: Different Approaches

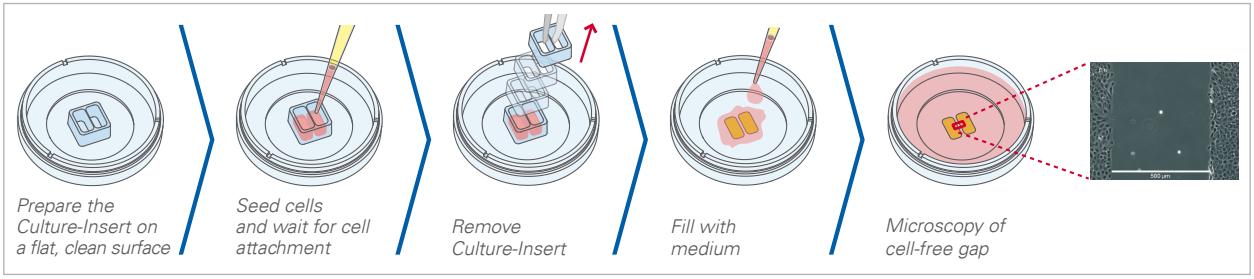
A gap in a cell monolayer can be created using different approaches:

- Insert: Physical cell exclusion is created by placing an insert/stencil on the culture surface before cell seeding
- Scratch: Mechanical cell removal is done by scratching the surface (scratch assay)
- Impedance: Electrical cell removal is achieved by using voltage application in a defined area

For further details and methods on how to create a gap, please have a look at this review:

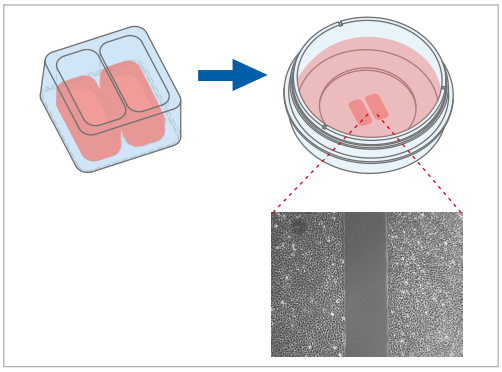
W.J. Ashby, A. Zijlstra. Established and novel methods of interrogating two-dimensional cell migration. Integr Biol 2012, 10.1039/c2ib20154b

Creating the Gap Using a Culture-Insert

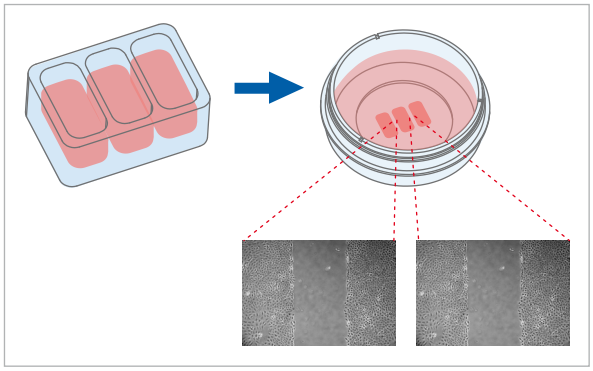


ibidi offers three different Culture-Inserts for gap creation: the ibidi Culture-Insert 2 Well, 3 Well, and 4 Well. Due to the specially designed bottom, the Culture-Inserts stick to the surface, preventing any cell growth beneath the walls. After the removal of the Culture-Insert, the newly created cell-free gap (wound) is clean without any remains. This method allows for the reproducible creation of highly defined gaps without any cells.

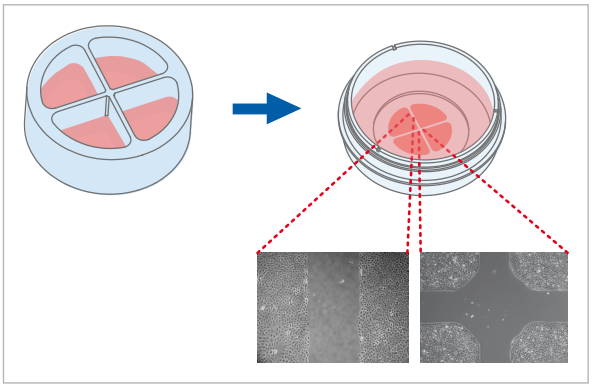
When placed on a flat, clean surface, the Culture-Insert 2 Well provides two reservoirs for culturing cells that are separated by a 500 µm thick wall. The cells are seeded in the reservoirs and cultured until they attach and form a monolayer. Removal of the silicone insert from the surface results in two precisely defined cell patches, which are separated by a zone that is exactly the same width as the separation wall. Cell migration can now be monitored by using live cell imaging or by taking photos at different time points.



The Culture-Insert 3 Well provides three cell culture reservoirs. It creates two cell-free gaps of 500 µm each and is, therefore, suitable for analyzing the migration of two technical replicates or different cell types.

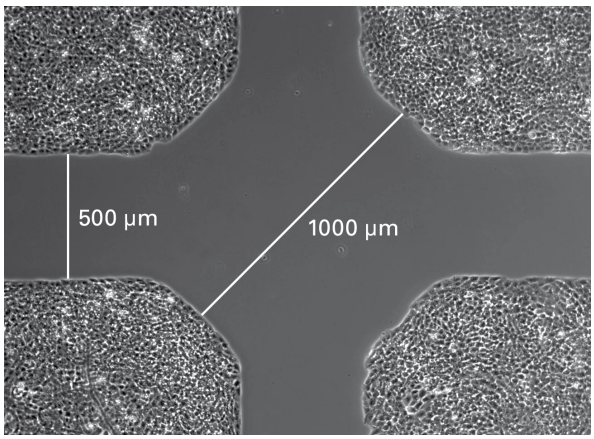


The Culture-Insert 4 Well provides four cell culture reservoirs. In addition to four 500 µm cell-free gaps, it includes a 1 mm center gap in the middle. The Culture-Insert 4 Well allows for observing the migration of up to four technical replicates or different cell types.



The Culture-Insert 3 Well | 4 Well both enable cell migration analysis after drug treatment or gene silencing/overexpression (e.g., by CRISPR/Cas9, siRNA, mRNA). Importantly, the non-treated control cells can be seeded and analyzed in the same vessel, sharing the same medium.

Unlike other gap creation techniques, the ibidi Culture-Inserts are suitable for 2D invasion assays and co-cultivation assays using two, three, or even four cell types or treatments at the same time.



Wound healing and migration assay using the ibidi Culture-Insert 4 Well.

Creating the Gap by Scratching

When doing a scratch assay, the cells are grown until they form a monolayer. Then, the cell surface is manually scratched with a pipet tip or a needle to generate a wound. Wound closure and cell migration are monitored by taking pictures at different time points or live cell imaging.

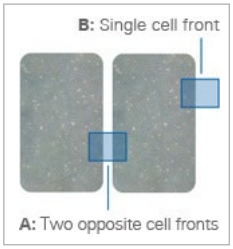
Regarding reproducibility, this method has certain drawbacks:

- The gap width is highly dependent on the pressure applied to the pipet tip and on its size.
- Scratching removes the surface coating in a non-reproducible way. This alters cell adherence and migration in this area.
- The removed cells form clumps of living and dead cells at the edges of the scratch in a non-reproducible way. The spreading of living cells can overlay the speed of migration.

Culture-Inserts vs. Scratch Assay

At first glance, the methods of scratching and placing a Culture-Insert seem to be two very similar approaches to create a cell-free gap. However, at a closer look, these two methods differ in important aspects that could influence the outcome of the assay:

	ibidi Culture-Inserts	Scratch assay
Gap creation	Cell seeding into designated areas	Scratching the cell surface with a needle or pipet tip
Gap size	Defined	Varying (i.e., not reproducible)
Gap surface residues	No	Extracellular matrix residues possible
Vessel surface damage	No	Yes, due to mechanical scratching on the surface
Cell damage	No	Yes, due to scratching the cells
Segregation & signaling of necrotic/apoptotic cells	Low	High
Internal reference *	Yes	No

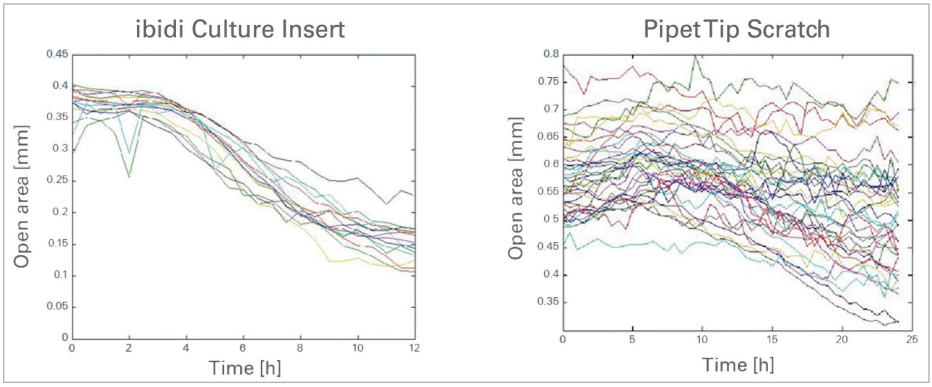


** The internal reference addresses the question of whether or not two opposite cell fronts influence each other. By using the Culture-Inserts, it is possible to measure the speed of:*

A: a cell front that is opposite another cell front; and

B: a single cell front that does not have an opposite cell front.

The ibidi Culture-Inserts Provide Improved Reproducibility When Compared With a Scratch Assay



Time-dependent changes of the open area due to cell migration. A comparison between gap creation by using the ibidi Culture-Insert 2 Well (left) and by scratching with a pipet tip (right).

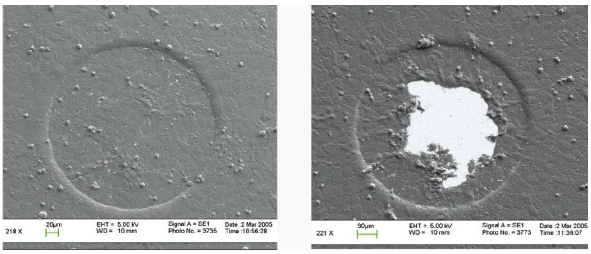
Data provided by M. Börries, University Freiburg, Germany.

Wound Healing Assays Using Impedance

The ECIS Wound Healing Assay replaces the traditional scratch assay. Instead of mechanically disrupting the cell layer with a pipet tip, and then following the migration of cells with a microscope, the ECIS System uses electric signals to both wound and then monitor the healing process.

The advantages of an impedance-based wound healing assay include:

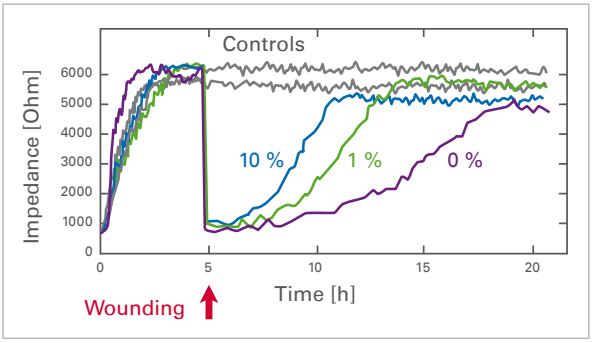
- The possibility to perform up to 96 simultaneous wound healing experiments
- The direct derivation of time constants and wound healing velocity
- The cost-efficiency of screening applications, from low to high throughput



The cell layer before (left) and after (right) electrical wounding. The circle marks the electrode area, in which the cells are exposed to electricity.

Experimental Example: The Healing Function of Serum

Electrical wounding was performed on a small population of cells that were in contact with a 250 µm electrode, thus creating a well-defined wound. The cell density within the wounded area is reflected by the impedance, which is continouosly measured by the ECIS system. After the wounding, the healthy cells around the electrode immediately migrate and replace the dead cells on the electrode, leading to changes in the measured impedance values. Depending on the fetal calf serum (FCS) concentration in the cell culture medium, the original cell density was once again reached after 10, 13, or 20 hours.



Impedance-based analysis of time-dependent wound closure using different FCS concentrations (0%, 1%, and 10%) in the cell culture medium.

More Information

Find detailed information about conducting a wound healing assay below:

Instructions

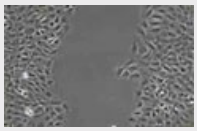
Instructions Culture-Insert 2 Well (PDF)

Application Notes

AN 21: Wound Healing Assay (PDF)
Setting up a wound healing assay with the ibidi Culture-Insert in a µ-Dish 35 mm.

AN 36: Wound Healing Assay in µ-Plate 24 Well (PDF)
A handling protocol for wound healing assays: Screening substances for pro- or anti-migrational effects.

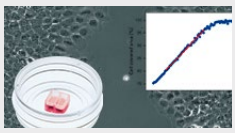
Movie:



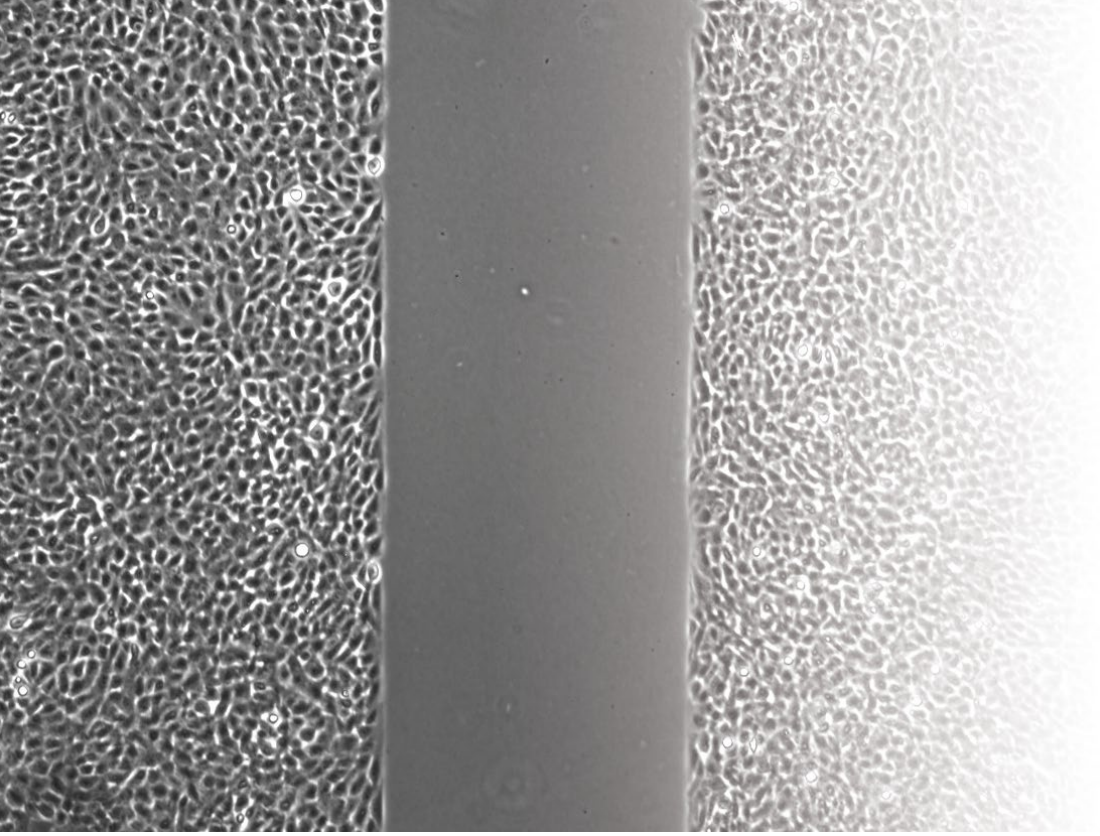
MV 16: Performing a Wound Healing Assay

Webinar:

For additional information, please also listen to ibidi's recorded webinar



"Cell Migration, Wound Healing, and Invasion: Performing an Assay and Quantitative Image Analysis".



cells in focus

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