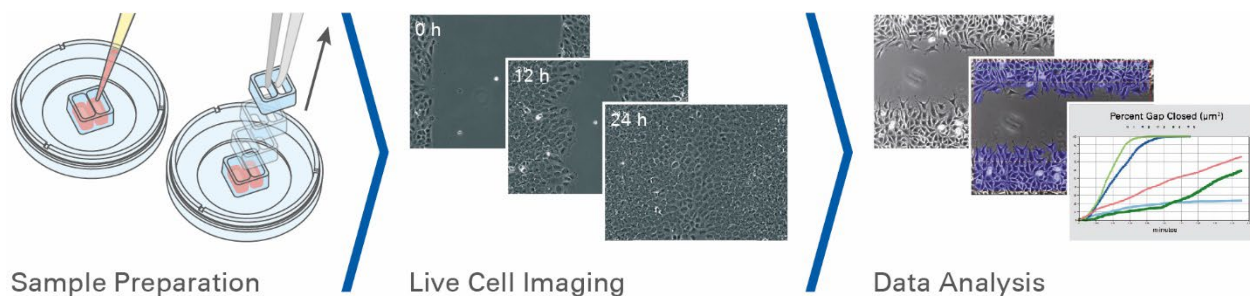
cells in focus ibidi

Wound Healing Assay
Using the ibidi Culture-Insert 2 Well in a µ-Dish $^{35\ mm}$

Cell migration plays a central role in many complex physiological and pathological processes. The wound healing assay is a simple method to study cell migration *in vitro*. This assay is based on the observation that when an artificial gap is created in a confluent cell monolayer, the cells at the edge of the gap begin to migrate until new cell-cell contacts are formed. The ibidi Culture-Insert family provides a complete solution for wound healing experiments, requiring only a few steps from sample preparation to image analysis.

This Application Note provides a detailed protocol for analyzing the migration behavior of MCF-7 cells using the ibidi Culture-Insert 2 Well. The Instructions of the Culture-Insert 2 Well provide more detailed information.



ibidi offers various solutions for wound healing assays:

- Culture-Insert 2 Well in µ-Dish $^{35\ mm,\ high}$ and µ-Dish $^{35\ mm,\ low}$
- Culture-Insert 2 Well in µ-Plate 24 Well
- Culture-Insert 3 Well in µ-Dish $^{35\ mm,\ high}$
- Culture-Insert 4 Well in µ-Dish $^{35\ mm,\ high}$
- Culture-Inserts for self-insertion in 2 Well, 3 Well, or 4 Well format

Related Documents:

- Application Note 36: Wound Healing Assay Using the Culture-Insert 2 Well in a µ-Plate 24 Well (PDF)
- Application Note 30: Optimizing Wound Healing and Cell Migration Assays (PDF)
- Application Note 67: Data Analysis of Wound Healing and Cell Migration Assays (PDF)

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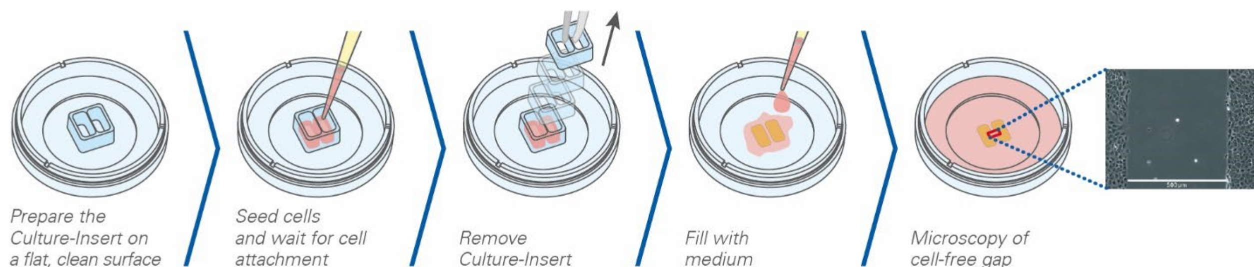
1 Material

- MCF-7 cells (ATCC: HTB-22; DSMZ: ACC115)
- Culture-Insert 2 Well in µ-Dish 35 mm, high (ibidi, 81176)
- RPMI cell culture medium (Sigma, R8758) + 10% FCS (Sigma, F0804)
- Trypsin-EDTA (Sigma, 59418C)
- Sterile tweezers
- Standard cell culture equipment (pipettes, tubes, sterile working bench, cell culture incubator, culture flasks, hemocytometer, etc.)
- Inverted microscope

2 Experimental Workflow

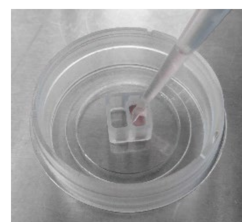


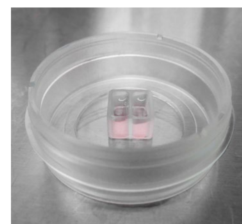
Experimental workflow for a wound healing assay using the Culture-Insert 2 Well.

2.1 Cell Seeding

Before starting the wound healing assay, one must consider experimental parameters such as cell type, growth rate, and incubation time. We recommend using a cell density that leads to a 100% optically confluent cell layer after 24 hours. Tips for optimizing your wound healing assay are available in Application Note 30: Optimizing Wound Healing and Cell Migration Assays.

Please read the Instructions before working with the ibidi Culture-Inserts. Perform all steps under sterile conditions. Before starting the experiment, prepare the cells in a standard cell culture flask (e.g., T75) with the cells adherent at the bottom. The cells should be healthy and optimally subconfluent on the day of the experiment.





1. Prepare the cell suspension as usual. Treat the cultured cells with trypsin (or the detachment reagent of your choice) for 5 minutes for detachment.
2. Stop the trypsin activity by adding cell culture medium. Harvest the cell suspension, centrifuge it, and dilute it in a low amount of culture medium for counting; the amount depends on the required cell concentration.
3. Count the cells. Counting should be performed as accurately as possible and always in the same way to have the same number of cells in all wells. Adjust the cell suspension to a final cell concentration of 3×10^5 cells/ml for most cell lines to obtain a confluent cell layer after 24 hours. However, consider the individual growth rates of different cell types.

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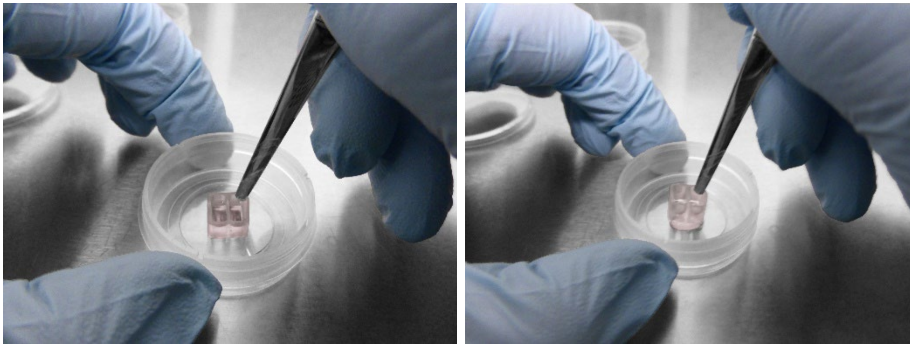
4. Apply 70 µl cell suspension into both wells of the Culture-Insert 2 Well. Avoid shaking the µ-Dish as this will result in an inhomogeneous cell distribution.
5. Incubate the cells at 37°C and 5% CO_2 for at least 24 hours. During incubation, ensure that the cells are at least at 90% humidity to avoid media evaporation.

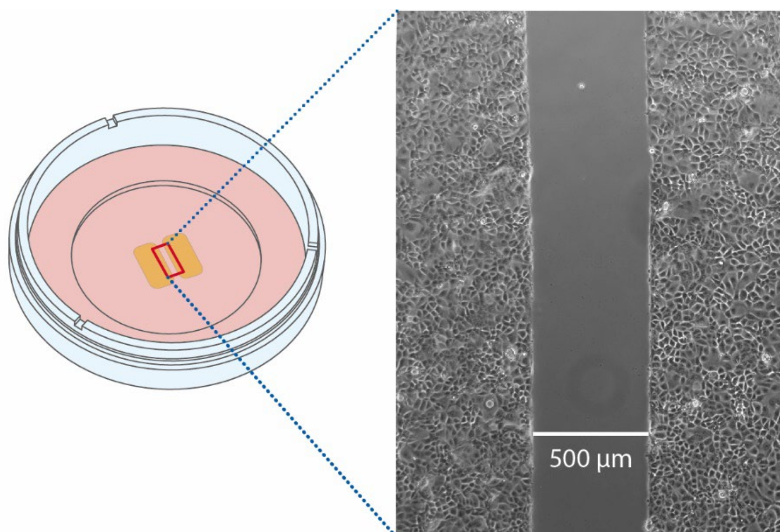
2.2 Gap Formation

1. A confluent cell layer is a prerequisite for starting a wound healing assay. Check the cell density after 24 hours under the microscope. If a 100% optically confluent cell layer is not achieved within 24 hours, return the µ-Dish with the Culture-Insert to the cell culture incubator and monitor the confluence regularly. If needed, change the medium for a longer incubation period.
2. When the desired confluency is achieved, use sterile tweezers to detach the Culture-Insert 2 Well from the µ-Dish by gently pulling on one corner.



Removal of the ibidi Culture-Insert 2 Well using sterile tweezers.

3. After removing the Culture-Insert, gently rinse the cell layer in the µ-Dish with cell-free medium or PBS to remove cell debris and non-attached cells.
4. Carefully fill the µ-Dish with 2 ml of pre-warmed cell-free medium (37°C) by slowly pipetting the medium from one edge of the dish. If necessary, supplement the medium with inhibiting or enhancing substances to evaluate their effects on the cell migration behavior.



Cell- and debris-free gap after removal of the ibidi Culture-Insert 2 Well with even cell fronts and a defined distance of 500 µm.

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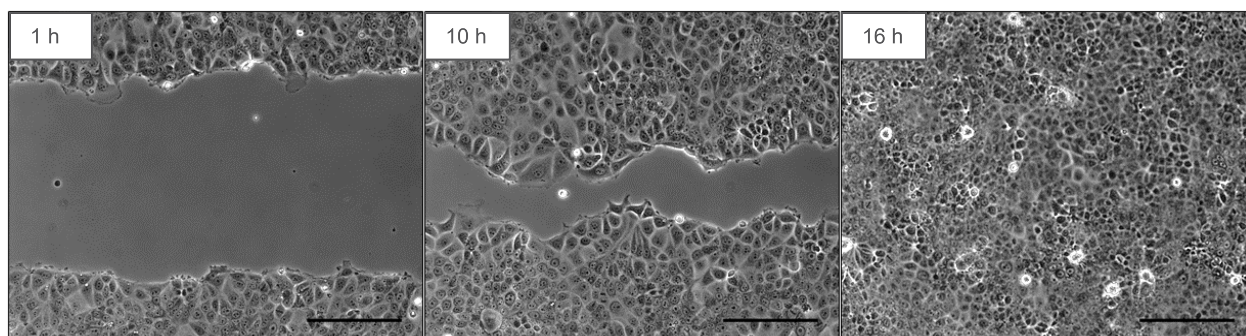
2.3 Image Acquisition

Migration and wound healing assays can be imaged either using brightfield or phase contrast microscopy for unlabeled cells or fluorescence microscopy when using a live stain. Data collection on the microscope can be performed manually or automatically.

We recommend recording a time-lapse video to determine the characteristics of the cell migration of the individual cell lines. During imaging, it is essential to maintain a stable and physiological environment to ensure natural cell behavior, which can be achieved using e.g., the ibidi Stage Top Incubators on top of an inverted microscope. These systems facilitate continuous monitoring of the same gap position throughout the experiment—a critical factor for accurate analysis.

Low magnification is adequate for most wound healing and cell migration experiments. Observing the widest possible field of view (FOV) is important to obtain the maximum amount of information. We recommend using 4x to 10x objectives for experiments using Culture-Inserts.

1. It is critical to begin monitoring cells immediately after creating the gap. Place the µ-Dish under the microscope and bring the gap into focus. To capture the 500 µm in one FOV, we recommend using a 4x or 10x objective lens. Here, the orientation of the gap area is not critical. However, for further analysis, orienting the gap horizontally or vertically is highly beneficial.
2. Start the acquisition process by capturing single frames with distinct time steps (e.g., 30–60 minutes) over several hours (e.g., 24 h), depending on the migration velocity and doubling time of the cell line used for the experiment.



Wound healing assay using the Culture-Insert 2 Well. Time-lapse phase contrast images of MCF-7 cells after removal of the Culture-Insert. Time-lapse measurements were performed over a period of 20 hours with a time interval of 30 minutes (scale bar: 200 µm).

2.4 Quantitative Image Analysis

Post-processing and analysis of the acquired time-lapse images can either be done manually using an image processing software (e.g., ImageJ) or by automated image analysis software.

Application Note 67: Data Analysis of Wound Healing and Cell Migration Assays provides detailed information about analyzing wound healing assay data.

Here, an exemplary analysis shows the process of gap closure by analyzing the cell-covered area over time.

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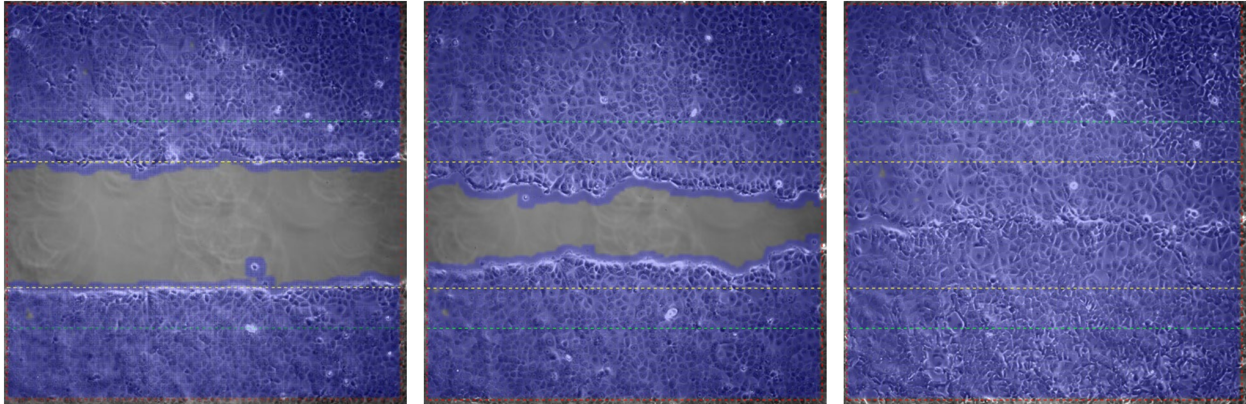

Quantitative image analysis of a wound healing experiment using an automated post-processing software. Violet represents the cell covered area and the yellow dotted line the 500 µm distance.

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