Wound Healing Assay
Using the ibidi Culture-Insert 2 Well in a µ-Dish 35 mm

1. General Information

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, upon the creation of an artificial gap on a confluent cell monolayer, the cells on the edge of the created gap will start migrating until new cell-cell contacts are established. The ibidi Culture-Insert family provides a complete solution for wound healing experiments, requiring only a few steps to go from sample preparation to image analysis.

This Application Note is a detailed protocol for analyzing the migration behavior of MCF-7 cells using the ibidi Culture-Insert 2 Well. More detailed information is provided in the Instructions of the Culture-Insert 2 Well.

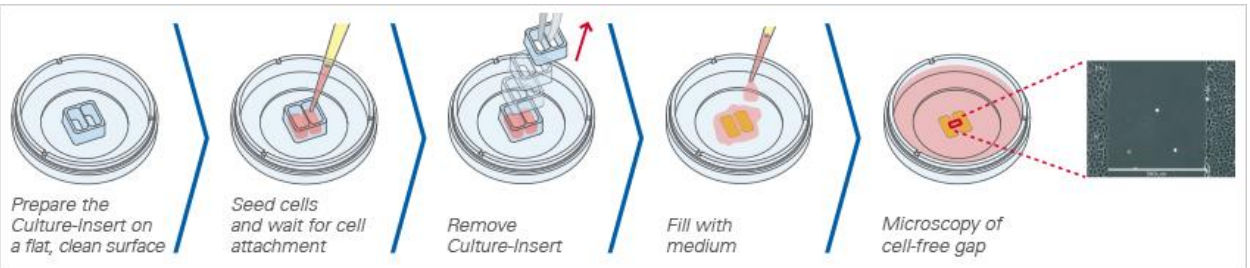

Figure 1 *Experimental workflow for a wound healing assay using the ibidi Culture-Insert 2 Well.*

Placed on a cell culture surface, the ibidi Culture-Insert 2 Well provides two cell culture reservoirs, each separated by a 500 µm wall. Filling cell suspension in both reservoirs allows cell growth in the designated areas only. Removing the Culture-Insert 2 Well after appropriate cell attachment creates a cell-free gap of approximately 500 µm. Microscopy is used to evaluate the wound healing process. Depending on your focus of interest, this can either be done by using video microscopy, or by observing images at distinct time points. Measuring the changes in cell covered area allows quantifying the speed of wound closure and provides cell migration characteristics.

The experimental workflow can also be applied to the Culture-Insert 3 Well and the Culture-Insert 4 Well. The only difference is the higher number of wells and the corresponding higher number of cell-free gaps.

2. Material

- Cells: MCF-7 (ATCC: HTB-22; DSMZ: ACC115)
- ibidi Labware: Culture-Insert 2 Well in µ-Dish $^{35\ mm,\ high}$ (ibidi, 80206)
- Cell culture surface: ibiTreat
- Cell culture medium: RPMI (Sigma, R8758) + 10% FCS (Sigma, F0804)
- Cell dissociation solution: Trypsin-ETDA (Sigma, 59418C)
- Sterile tweezers
- Inverted microscope, preferable with an automated image acquisition system and stage top incubator for live cell imaging

3. Experimental Workflow

3.1. Step 1: Cell Seeding

In order to establish a reliable data acquisition system, you have to define the experimental parameters before starting the experiment. This includes for example the choice of the right cell seeding density. We recommend using a density that leads to a 100% optically confluent cell layer after 24 hours.

1. Prepare your cell suspension as usual. It is recommended to include a centrifugation step to remove dead cells and cell debris. Adjust the cell suspension to a cell concentration of ca. $3x10^5$ cells/ml to obtain a confluent cell layer after 24 hours.
2. Apply 70 µl cell suspension into each well of the Culture-Insert 2 Well. Avoid shaking the µ-Dish as this will result in an inhomogeneous cell distribution.
3. Incubate your cells at 37 °C and 5% CO_2 for at least 24 hours.

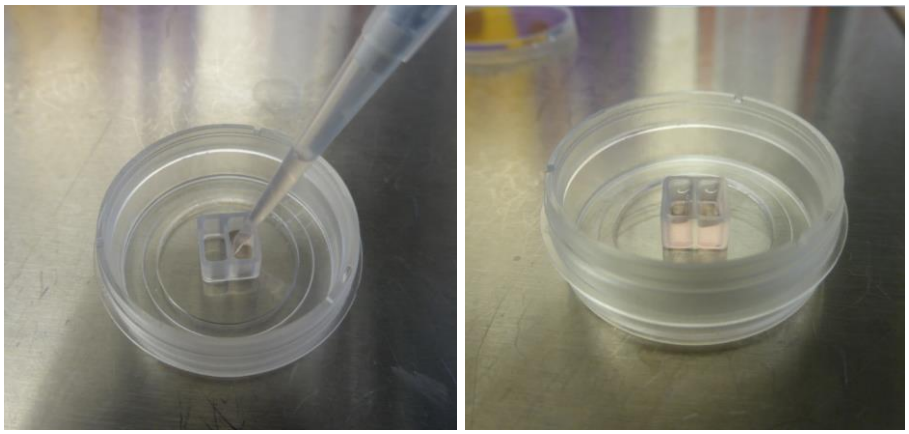

Figure 2 *Seeding cells in the ibidi Culture-Insert 2 Well.*

3.2. Step 2: Gap Formation

A confluent cell layer is a prerequisite for starting this assay. Fresh medium is added after the removal of the Culture-Insert 2 Well. If necessary, supplement your medium with inhibiting or enhancing substances for evaluating their effects on the migration behavior of the cells.

1. Check the cell density after 24 hours under the microscope. In case that no confluent cell layer is reached after 24 hours place the µ-Dish again in the cell culture incubator for another 12–24 hours. Check the confluence regularly.
2. Gently remove the Culture-Insert 2 Well with sterile tweezers. For removing the Insert grab one corner as shown in Figure 3.

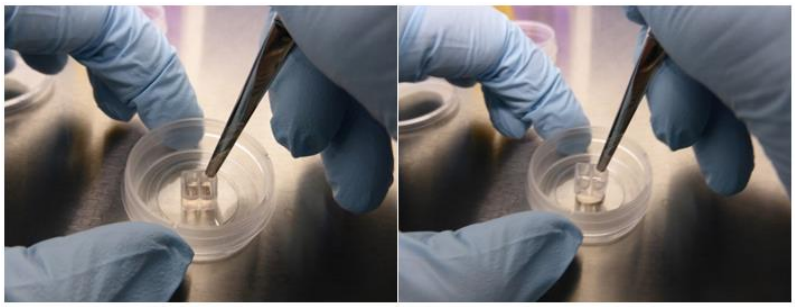

Figure 2 *Removal of the ibidi Culture-Insert 2 Well using sterile tweezers.*

3. After removing the Insert, check whether your cell layer is still attached to the surface of the µ-Dish.
4. Wash your cell layer with cell-free medium or PBS to remove cell debris and non-attached cells.
5. Fill the µ-Dish with cell-free medium applying the recommended volume of 2 ml.

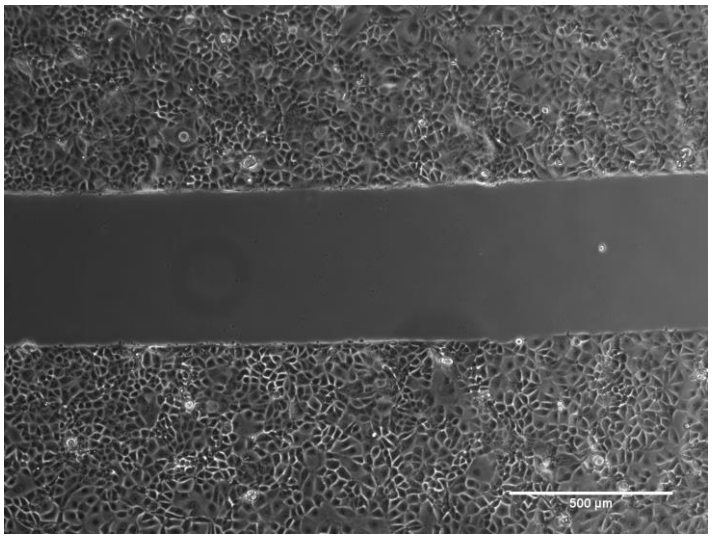

Figure 3 *Cell-free gap created by the ibidi Culture-Insert 2 Well.*

3.3. Step 3: Acquisition of Microscopy Images

We recommend recording a time-lapse video to determine the time dependency and the characteristics of the cell migration.

1. Place the dish on the microscope and move it until you have the gap and both cell fronts captured in the image. Use a 4x/5x or a 10x objective lens. The orientation of the wound area is not critical, but needs to be horizontal or vertical.
2. Start the observation process by taking images several times throughout the following hours.
3. Time lapse measurements for MCF-7 cells cultured on the ibiTreat surface were performed for 20 hours with a time interval of 30 minutes.

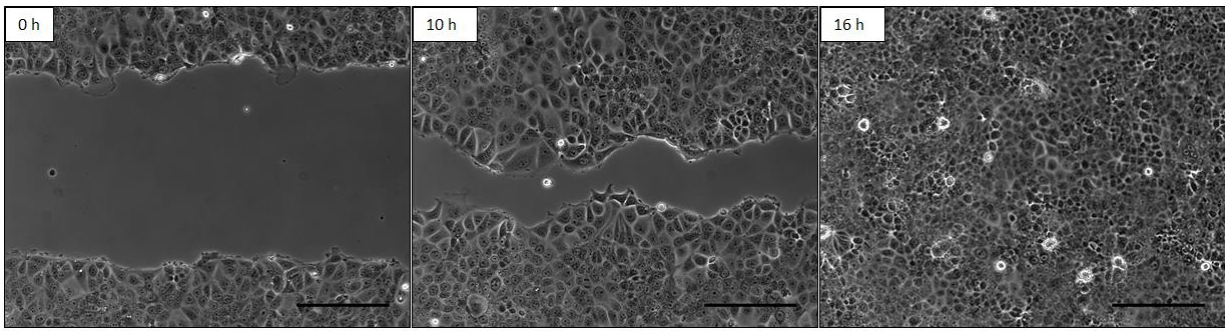

Figure 4 *Time lapse measurement of a migration assay using MCF-7 cells (scale bar: 200 µm).*

3.4. Step 4: Quantitative Image Analysis with FastTrack AI and Data Interpretation

The microscopic pictures have to be analyzed for obtaining information about the migration characteristics of the cultured cells. This can either be done manually by using an image processing software, or by automated image analysis using the Wound Healing FastTrack AI Image Analysis provided by ibidi.

The example data shown here was analyzed using FastTrack AI. The automated image analysis detects the cell covered area. Plotting the cell covered area against the time showed the process of gap closure. The linear phase can be used to characterize the migration (= speed of wound closure).

The slope of the linear phase revealed an average scratch closure speed of 0.0184*106 µm²/hour (=0.44*106 µm²/day).

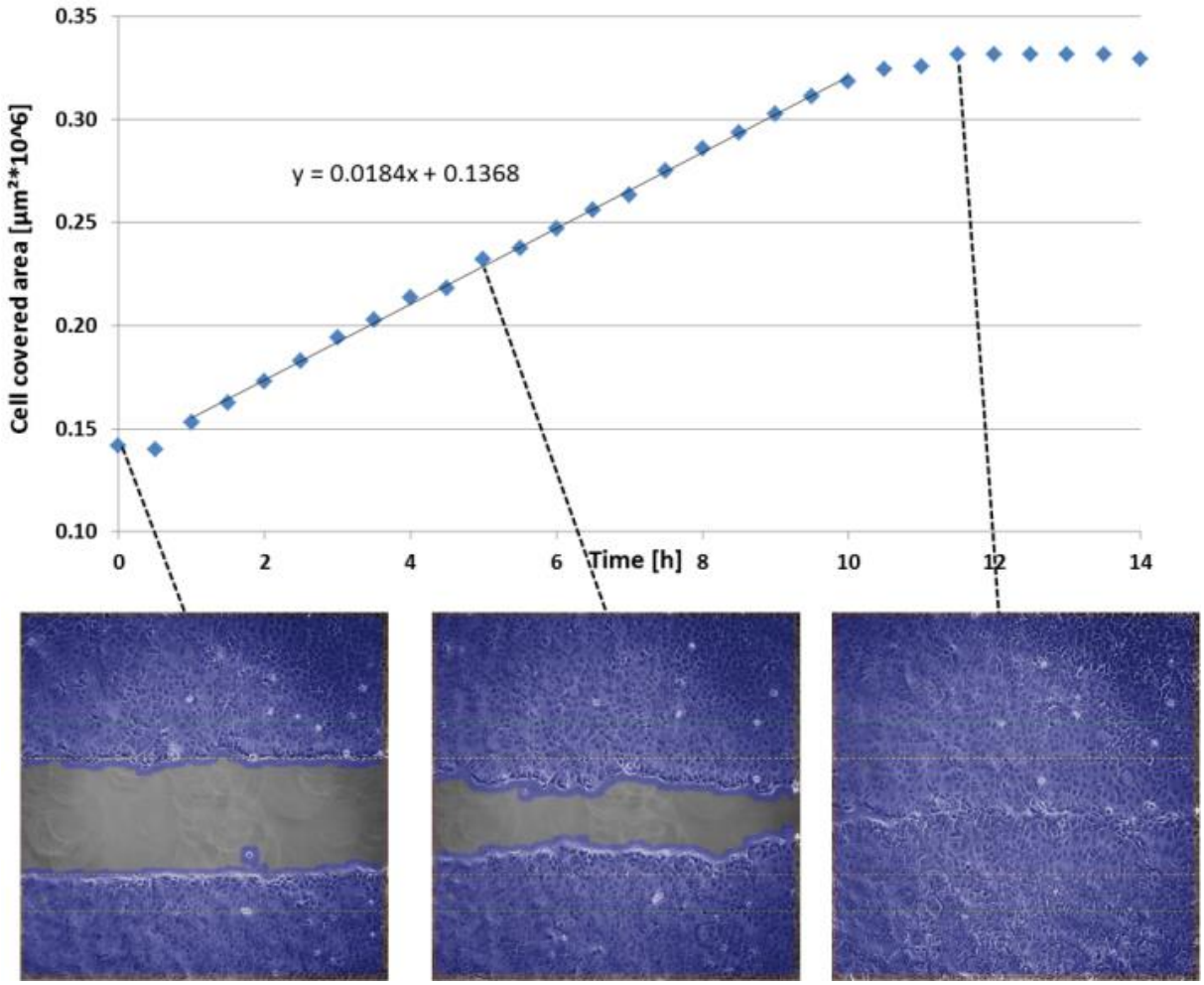

Figure 5 *Quantitative image analysis of a wound healing experiment by FastTrack AI.*