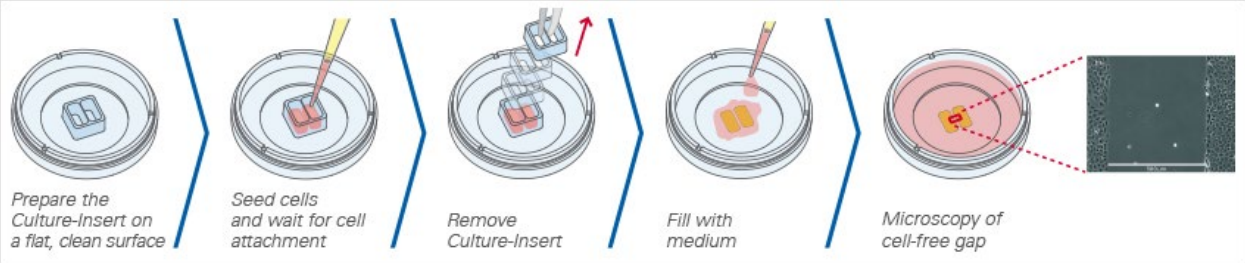
Optimizing Wound Healing and Cell Migration Assays

Wound healing and cell migration studies serve as a cornerstone for unraveling complex systems in health and disease. These assays are a straightforward but powerful tool for studying cell migration kinetics.

The initial step usually involves creating a gap in a confluent cell layer. For instance, a gap can be achieved by cultivating cells on neighboring growth surfaces separated by a barrier.

Then, tracking the closure of this gap via time-lapse imaging offers valuable data on cell movement. This approach enables researchers to examine the effects of specific genes, physiological conditions, or pharmaceutical agents on key migration parameters such as speed, directional focus, and overall gap closure.

Obtaining accurate and reliable results comes from a well-designed experimental setup. This Application Note offers an overview of crucial assay parameters and guidelines for planning effective wound healing and cell migration assays.



Principle of wound healing and migration assays.

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

Find more detailed experimental instructions in:

- Application Note 36: “Wound Healing Assay Using the Culture-Insert 2 Well in a µ-Plate 24 Well” (PDF)
- Application Note 21: “Wound Healing Assay Using the ibidi Culture-Insert 2 Well in a µ-Dish 35 mm ” (PDF)
- Application Note 67: “Data Analysis of Wound Healing and Cell Migration Assays” (PDF)

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1 Planning the Experiment

Before starting with wound healing and cell migration experiments, the optimal experiment setup must be determined empirically. The chosen system should align closely with your research objectives and allow for feasible data collection. The key factors to consider will be discussed in the next chapters.

1.1 Cell Type and Passage Number

Selecting the **optimal cell type** is crucial in wound healing experiments, guided by the research focus. Choices range from primary cells, which mimic *in vivo* conditions but are challenging to culture, to easier-to-handle cell lines that may exhibit altered behaviors.

Repeated culturing can lead to changes in cells, affecting key experimental outcomes, such as migration speed and response to stimuli. Consistency in **passage number** is vital for reliable and interpretable data, as higher passage cells often show slower growth and increased mortality, complicating results.

1.2 Cell Density When Creating the Gap

In wound healing assays, it is crucial to establish a **confluent cell layer** suitable for creating a gap to study cell migration. While the density should be sufficient to form this confluent layer, overly dense cultures could lead to issues, such as altered cellular behavior or detachment of cell layer patches when creating the gap. Therefore, the aim is not just to reach a confluent state but to do so at a density that allows for healthy cell behavior and accurate experimental outcomes. Additionally, researchers often use a low dose of the DNA cross-linker mitomycin C (10 µg/mL), which inhibits proliferation, enabling a more precise focus on migration.

We recommend using a cell density that results in a confluent cell layer 24 h after cell seeding. Depending on your cell type, add a seeding concentration of 3–7 × 10^5 cells/ml when using ibidi Culture-Inserts.

1.3 Positive and Negative Controls

Incorporating controls is crucial for meaningful, reliable results in cell migration experiments. A positive control, such as an epidermal growth factor (EGF) medium, should be used to stimulate cell migration and to establish a reference value for the response to your bioactive substances. A negative control, typically a medium without bioactive compounds, is necessary to determine the baseline migration of your cell culture.

Technical replicates should always be included to compensate for the variability of the experimental conditions. **Biological replicates**, ideally using separate cell populations from different individuals, compensate for batch effects and the inherent biological variability. Note: Dividing a single cell culture into multiple aliquots does not create true biological replicates, as these cells are not independent and share a common history. Employing distinct cell populations ensures statistical robustness and strengthens the reliability of your data.

1.4 Medium Composition

The cell culture medium is vital in wound healing assays, providing key nutrients and influencing cell behavior. Choosing the optimal medium ensures cell health and an accurate representation of the biological process. Factors like growth factor concentration and serum content are crucial in this selection.

To effectively study bioactive compounds in wound healing assays, it is necessary to establish their ideal concentrations. Finding these concentrations is best achieved through a **dose-response curve**, which helps to find a balance between the desired cellular response and avoiding cytotoxicity. First, existing literature or recommendations from the compound's manufacturer should be reviewed. Then, the concentration should be optimized based on how it affects cell morphology, movement, and survival.

1.5 Gap Creation Method

The method of creating a cell-free gap is a crucial factor in wound healing assays, directly influencing cellular behavior and the reproducibility of experimental data. Traditional techniques include mechanical scratching with a pipet tip ("**scratch assay**") and **wound burning** with electrodes. One advantage of these methods is the generation of damage-associated molecular patterns (DAMPs) from damaged cells, creating an *in vivo*-like wound environment. However, these techniques, especially the scratching method, often result in inconsistent gap sizes and cell layer damage, complicating data interpretation.

In contrast, the ibidi Culture-Inserts offer multiple advantages for reproducible experiments. They allow for a **precisely defined 500 µm cell-free gap**, ensuring no leakage during cultivation and leaving no residual material post-removal. These features contribute to highly consistent and reliable data, making the Culture-Inserts ideal for high-throughput applications. Their design offers the flexibility of seeding different cell types into each well, allowing for comparative studies on invasional behavior. Additionally, ibidi Culture-Inserts minimize the generation of damage-associated molecular patterns (DAMPs) from damaged cells. Therefore, it is possible to analyze the influence of DAMPs on the migrational behavior.

1.6 Imaging

Imaging plays a pivotal role in wound healing assays, as it allows for the detailed observation and analysis of cell migration in real-time. During imaging, a stable and physiological environment should be maintained to ensure natural cell behavior, which can be achieved using the ibidi Stage Top Incubation Systems. These systems ensure that cells are maintained in optimal conditions during the whole wound healing assay, facilitating continuous monitoring of the same gap position throughout the experiment—a critical factor for accurate analysis.

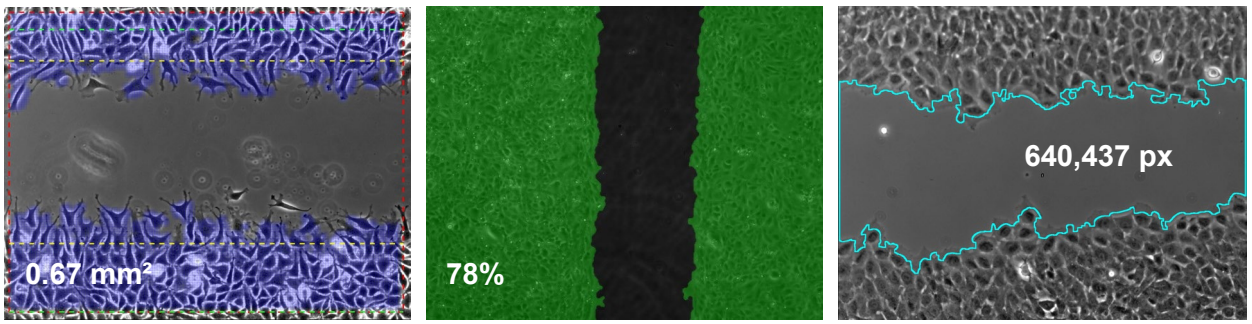
A low magnification is adequate for most wound healing and cell migration experiments. Observing the widest possible field of vision is important to obtain the maximum amount of information. We recommend using 4x to 10x objectives for experiments with the ibidi Culture-Inserts.

It is critical to begin monitoring cells immediately after the creation of the gap, typically employing phase contrast microscopy to visualize cell dynamics without the need for staining. For robust analysis, images of the gap should be captured at consistent intervals (e.g., at 30-min intervals post-wounding). It is generally sufficient to monitor the gap closure for up to 24 h. If fluorescence microscopy is used, it is recommended to double check for phototoxicity using control experiments.

2 Considerations for Data Acquisition

2.1 Output Parameters of Wound Healing and Cell Migration Assays

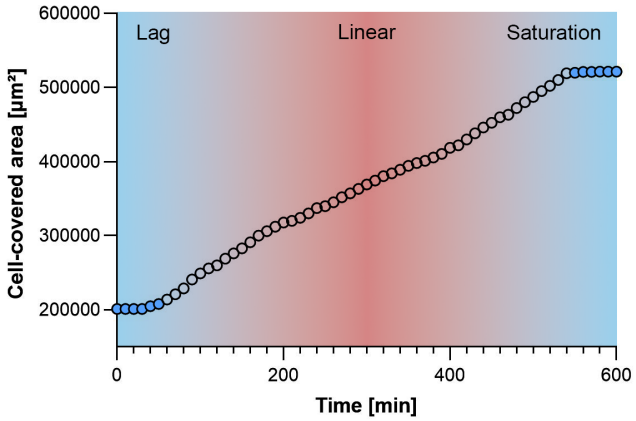
In wound healing assays, the **initial gap width** is measured right after the gap is created, setting a baseline for comparison. Measurements of the **cell-covered area** (alternatively: surface coverage area) are then consistently taken at regular intervals to monitor the reduction in gap width. The key output parameter is the **rate of gap closure** (alternative names: e.g., speed of wound healing, gap area coverage rate, wound closure rate), which can be quantified both as a percentage of the initial gap closed and in absolute values (µm² or pixels) over time. The use of image analysis software, such as ImageJ, enhances the precision and efficiency of these measurements.



Exemplary gap width and gap closure evaluations. (Left) Total area of the cell-free gap in mm². (Center) Proportion of the cell-covered area in %. (Right) Total area of the cell-free gap in pixel sum.

2.2 Choosing Optimal Time Points for Analysis

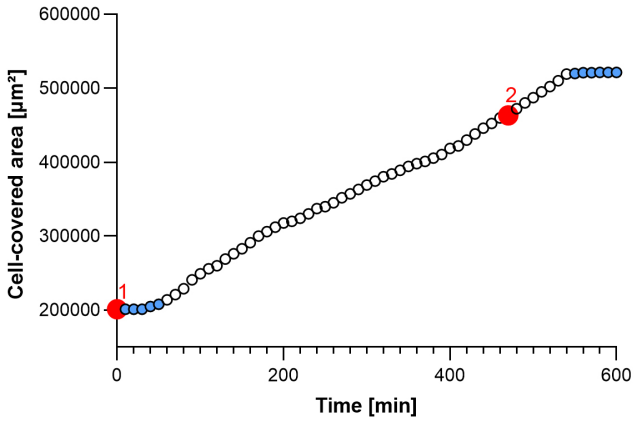
Before image analysis and subsequent quantification, it is essential to understand the typical gap closure curve of wound healing and cell migration experiments. Upon creating the gap, cells may exhibit a **lag phase** before migration begins, though this can be brief or absent, leading directly into the **linear phase**. The linear phase, in which the rate of gap closure remains constant, is crucial for data collection and is the most meaningful phase of the assay. As the cells approach confluence, the rate of gap closure decreases, causing the cells to enter the **saturation phase**.



Plot visualizing a typical differential surface coverage. The blue areas indicate an inhibited change in surface coverage, and the red area indicates the linear phase of the differential surface coverage.

2.2.1 Two-Point Measurement

The simplest method for tracking migration is the two-point measurement, where the first image is taken immediately after gap formation (start point) and a second time (end point) after a predetermined interval. The end point should be within the linear gap closure rate phase to ensure accuracy. Determining the second time point requires a preliminary test that should be carried out under optimal conditions to determine the duration of the linear phase. Ideally, the end point should be just before the curve reaches the saturation phase, where the gap closure rate starts to decline.

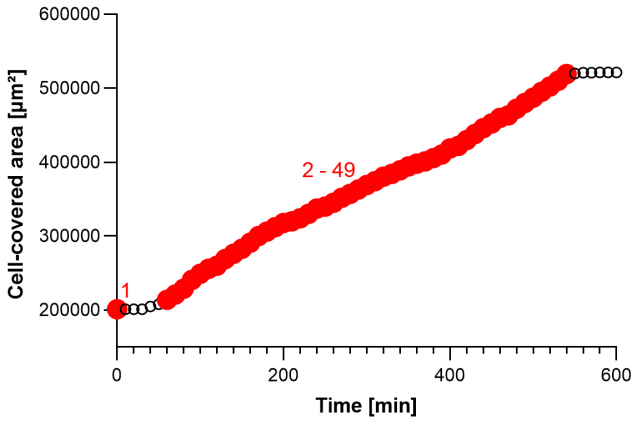


Plot visualizing a typical differential surface coverage with (1) the start point and (2) the end point of the two-point measurement.

2.2.2 Ten-Point (or More) Measurement

For a more detailed analysis, capturing ten or more images throughout the assay can provide a comprehensive view of the migration curve. This method unveils subtler aspects of the treatments, such as variations in the **slope of the linear phase**, changes in the lag phase duration, or the precise timing of gap closure. The exact determination of the linear phase enables the calculation of the **cell front velocity.**

Please refer to Application Note 67: “Data Analysis of Wound Healing and Cell Migration Assays” (PDF) for more information on calculating the rate of gap closure and cell front velocity.



Plot visualizing a typical differential surface coverage with (1) the start point and (2–49) the other time points of the multi-point measurement.