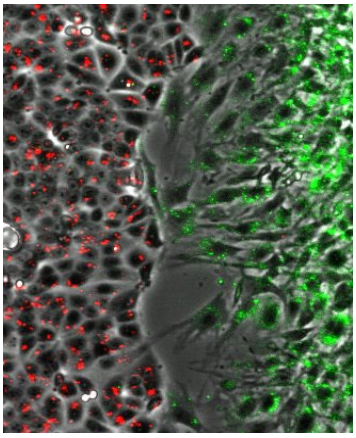
Co-Culture Invasion Assay Using the Culture-Insert 2 Well and the Live Cell Labeling Kit Cellaris™

Tracking fluorescent-labeled cells over an elongated period in invasion assays can be highly beneficial in investigating the migration behavior of cell populations. When using native cell lines that do not express a reporter dye, the cells must be labeled transiently. Unfortunately, many transient dyes influence cell behavior and are only suitable for short-term microscopy images, as their intensity decreases due to degradation, photobleaching, or cell division. Ultra-bright fluorescent nanoparticles offer a promising new approach. After a short incubation period of a few hours, the cells are packed with biocompatible and non-toxic fluorescent nanoparticles and can be imaged continuously for longitudinal studies.

In this application note, two different cell lines were labeled with Cellaris™ fluorescent nanoparticles (Luminicell) and then seeded in a Culture-Insert 2 Well for a 2D invasion experiment. The fluorescent labeling helped to distinguish the migration behavior of the two cell populations after removing the Culture-Insert 2 Well.



Co-culture of MCF-7 cells (Cellaris™ 670, red) and NIH-3T3 cells (Cellaris™ 540, green), grown in the Culture-Insert 2 Well.

ibidi offers various solutions for migration assays:

- Culture-Insert 2 Well in µ-Dish 35 mm
- Culture-Insert 3 Well in µ-Dish 35 mm
- Culture-Insert 4 Well in µ-Dish 35 mm
- micro-Insert 3D in µ-Dish 35 mm

Related Documents:

- AN 21: Wound Healing Assay Using the ibidi Culture-Insert 2 Well in a µ-Dish 35 mm, high (PDF)
- AN 30: Optimizing Wound Healing and Cell Migration Assays (PDF)
- AN 67: Data Analysis of Wound Healing and Cell Migration Assays (PDF)

Keywords:

Migration, Wound Healing, Invasion, Co-Culture, Live Cell Imaging, Live Cell Stain, Live Cell Fluorescence, Nanoparticles

1 Material

1.1 Reagents and Buffers

- Cellaris™ 540 - Cell Labelling Kit (Green), and Cellaris™ 670 - Cell Labelling Kit (Red), (Luminicell)
- MCF-7 cell line (breast cancer, human) and NIH-3T3 cell line (fibroblast, mouse)
- Cell culture medium: RPMI-1640 (Gibco, 21845034), supplemented with 10 % fetal bovine serum (Gibco; 10270106)
- Standard cell culture reagents (PBS, Trypsin/EDTA)

1.2 Equipment

- Culture-Insert 2 Well in µ-Dish 35 mm (81176)
- Standard cell culture equipment (sterile working bench, cell culture incubator, culture flasks, pipets, tips, etc.)
- Tweezers
- Nikon TI microscope equipped with FITC and Cy5 HC filter sets (Nikon), 4x CFI PlanFluor DL objective (Nikon), LED Sola Light Engine (lumencor), ORCA-Flash 4.0-LT camera (Hamamatsu Photonics)
- ibidi Stage Top Incubator Slide/Dish, CO_2/O_2 – Silver Line (12722)

2 Preparation

2.1 Labeling Cells With the Cellaris™ Cell Labeling Kit

- Cultivate cells in a T25 cell culture flasks (or other cell culture labware) to 80–90 % confluency.
- Prepare the staining solutions seperately by diluting the Cellaris™ nanoparticles to 2 nM (100 x) in 5 ml supplemented cell culture medium.
- Replace old medium in cell culture flasks with the prepared staining solution, and incubate in cell culture incubator for 1–24 hours, according to cell type. Here, we stained the MCF-7 cells with Cellaris™ 670, and NIH-3T3 with Cellaris™ 540, for 2–4 hours.

2.2 Setting up the Wound Healing Assay in Culture-Insert 2 Well

- After staining, harvest cells as usual. Include a centrifugation step to remove cell debris and free nanoparticles.
- Prepare cell suspension of 3–7 × 10^5 cells/ml, according to your cell type. For MCF-7 and NIH-3T3, cell concentration of 3 × 10^5 cells/ml was used, resulting in a confluent cell layer within 24 hours.
- Unpack the µ-Dish with the Culture-Insert 2 Well in the sterile working bench.
- Apply 70 µl of MCF-7 suspension in one well, and 70 µl of the NIH-3T3 in the second well of the Culture-Insert. Let cells settle down for 5–10 minutes and avoid shaking as this will result in inhomogeneous cell distribution.

- Place the µ-Dish with the Culture Insert in a humid chamber (e.g., a Petri dish with a piece of wet tissue) and place it in the cell culture incubator. Incubate at 37 °C and 5 % CO_2 for 24 hours or until the cell layers are confluent.
- Place the µ-Dish in a sterile working bench and gently remove the Culture-Insert 2 Well using a sterile tweezer, grabbing a corner of the Culture-Insert 2 Well.
- Fill the µ-Dish with 2 ml supplemented cell culture medium. If necessary, a washing step can help remove non-adherent cells or cell debris.
- Close the µ-Dish with the lid. The probe is ready for imaging.

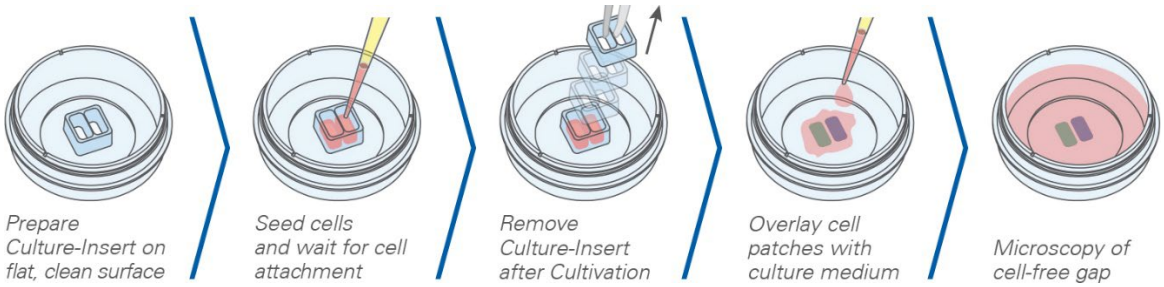


Figure 1 *Culture-Insert 2 Well workflow*

2.3 Live Cell Fluorescence Microscopy

- Heat up your microscope's incubation system (e.g., ibidi Stage Top Incubator) in advance (at least 0.5–2 h before the experiment). Perform the experiment at 37 °C, 5 % CO_2, and 80 % humidity.
- Place the probe in the incubation chamber on the microscope stage and focus your cell layer with the 4x objective.
- Set up the experiment: take an image of the wound in phase contrast, FITC (Cellaris™ 540), and Cy5 (Cellaris™ 670) channels every 20 minutes. Make sure the illumination is turned off in the time between image acquisition.
    - Cellaris™ 540 is excitable with violet/blue light source (Ex 423 nm, Em 540 nm).
    - Cellaris™ 670 is excitable with blue/green light source (Ex 506 nm, Em 670 nm).

Important Note: To reduce the illumination stress on cells, it is recommended to decrease the illumination intensity of the excitation source, for example by inserting the neutral density filters (ND4, ND8) into the light pathway.

- Monitor the cell behavior for 24–72 hours. Process and evaluate the time-lapse images with suitable image analysis software, such as ImageJ (NIH).

3 Results

The staining procedure effectively incorporated the nanoparticles into the cells without noticeable adverse effects on cell viability. Cellaris™ provided clear and distinguishable fluorescence signals for time-lapse microscopy. The Culture-Insert 2 Well enabled the creation of a clear wound gap, for precise tracking of cell migration and behavior over time of the two individually labeled cell populations as shown in Figure 2. Observing cell movement in real-time under live cell imaging conditions provides valuable insights into the dynamics of wound closure, which is clearly visible in Figure 2.

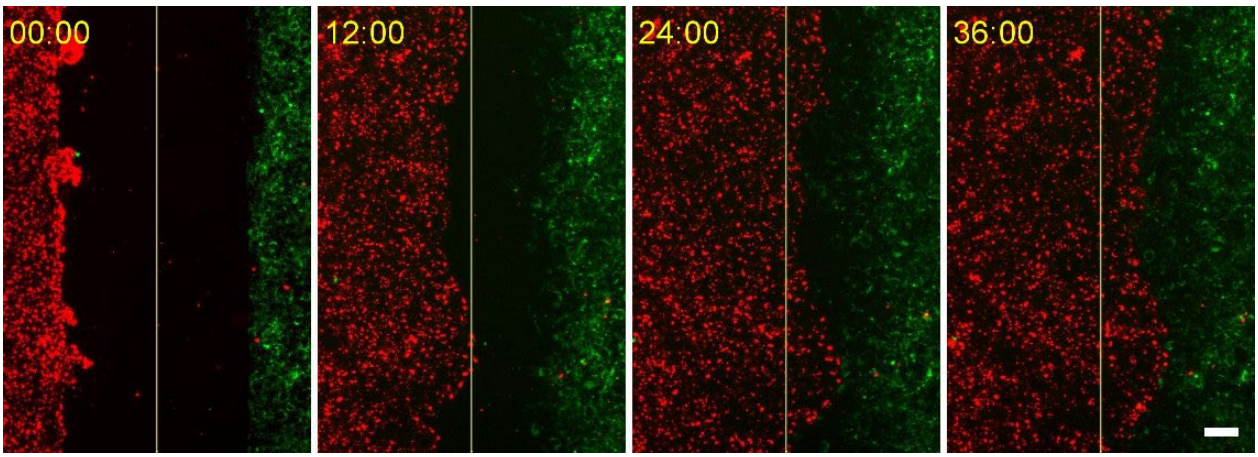


Figure 2 *Time-lapse images of MCF-7 cells (Cellaris™ 670, red) and NIH-3T3 cells (Cellaris™ 540, green) show the migration of the respective cell line and closure of the initially 500 µm wide wound gap. The yellow line marks the middle of the gap. The MCF-7 cells migrated faster and closed the gap within 36 hours. Scale bar = 100 µm*

The combination of phase contrast and fluorescence imaging enabled the visualization of labeling homogeneity. Figure 3 shows, that the particles are not just homogeneous within one cell but homogeneous over the cell layer. Additionally, there appears to be no cross-labeling between the two cell fronts when they merge, as indicated by the white arrows. This evidence shows that Cellaris™ exhibits no crosstalk effects in multi-colored experiments, even over long-term live cell studies.

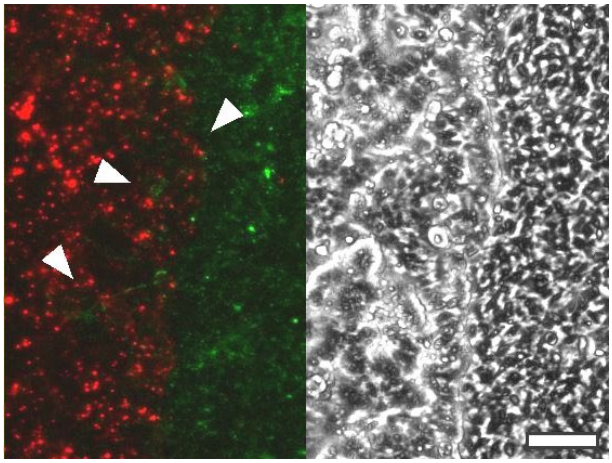


Figure 3 *Detail of a merged image of the MCF-7 (Cellaris™ 670, red) and NIH-3T3 (Cellaris™ 540, green) taken 90 hours after start of the experiment. Arrows show cells invading into the other population. Right image shows the same area in phase contrast. Scale bar = 100 µm*

The aim of this study was to explore co-culture and migration studies by using a simple but effective labeling method. MCF-7 and NIH-3T3 cells were successfully labeled using the Cellaris™ Cell Labeling Kit and subsequently used for an invasion wound healing assay with the Culture-Insert 2 Well. Ease of use of both Cellaris™ Cell Labelling Kits and the Culture-Insert 2 Well, allow for easy experiments with reliable and reproducible results.