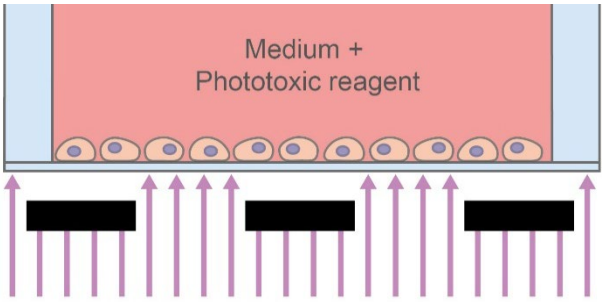
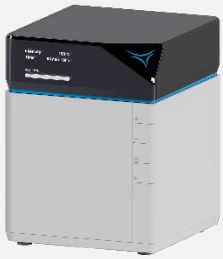
Photo-Induced Cell Migration Using the ibidi Micro Illumination System

The ibidi Micro Illumination System, when used in combination with photoinitiators activated by 365 nm light, can serve as a phototoxic tool for spatially defined cell killing or wound healing assays. In this configuration, the photoinitiators respond to the light exposure by inducing localized cellular stress and cell death, thereby restricting damage to the precisely illuminated area. The resulting mode of cell death—necrosis or apoptosis—depends on several factors, including the cell line, illumination dose, and the specific phototoxin employed. The migration and proliferation of the surrounding untreated cells subsequently repopulate the damaged or cell-free regions. Depending on the pattern of these regions, various migration assays can be established (e.g., scratch-like migration assays or array-based formats for high-throughput screening).



This Application Note describes a protocol for photo-induced wounding and cell migration and shows examples with different phototoxins and cell types.

ibidi Solutions for Photo-Induced Cell Migration



- ibidi Micro Illumination System
- µ-Slide VI 0.4 ibiTreat
- ibidi Pump System

Related Documents

- Instructions ibidi Micro Illumination System (PDF)
- Instructions µ-Slide VI 0.4 (PDF)
- Instructions ibidi Pump System (PDF)
- Application Note 03: Cell Culture in ibidi Channel Slides Using the µ-Slide VI 0.4 as an Example (PDF)
- Application Note 08: Coating Protocols for ibidi Labware (PDF)
- Application Note 13: Endothelial Cell Culture Under Perfusion With the ibidi Pump System and the µ-Slide I 0.6 Luer (PDF)
- Application Note 31: Serial Connection of µ-Slide VI 0.4 Channels for Flow Experiments (PDF)
- Application Note 72: RGD Micropatterning Using the ibidi Micro Illumination System for Spheroid Generation and Cultivation (PDF)
- Application Note 73: 2D Whole Protein Pattern Based on a PLL-PEG-Passivated Coverslip Surface Using the ibidi Micro Illumination System (PDF)
- Application Note 74: 3D Hydrogel Constriction in the µ-Slide I Luer Using the ibidi Micro Illumination System (PDF)
- Application Note 75: Structuring a Photoresist-Coated Wafer With Photolithography Using the ibidi Micro Illumination System (PDF)
- Photomask Templates (ZIP)

1 Material

1.1 Reagents and Buffers

- Mouse embryo fibroblasts (NIH-3T3, ACC 59, DSMZ)
- Rat embryo fibroblasts (Rat1)
- DMEM with L-glutamine (41965039, Gibco) + 10% FCS (10270106, Gibco)
- Human umbilical vein endothelial cells (HUVEC, C-12203, PromoCell)
- Endothelial cell growth medium (C-22010, PromoCell)
- Lithium-phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) (900889, Sigma-Aldrich)
- 2-Hydroxy-4-(2-hydroxyethoxy)-α-methylpropiophenone (Irgacure) (410896, Sigma-Aldrich)
- Collagen IV (354233, Corning®)
- Matrigel® basement membrane matrix (356234, Corning®)
- PBS (14190144, Gibco)
- Sterile, ultrapure water

1.2 Equipment

- ibidi Micro Illumination System (76000, ibidi)
- 3” photomask (compugraphics, MacDermid Alpha)
- µ-Slide VI $^{0.4}$ ibiTreat (80606, ibidi)
- ibidi Pump System (10902, ibidi)
- Perfusion Set YELLOW-GREEN, 50 cm, ID 1.6 mm, 10 ml Reservoir (10964, ibidi)
- Serial Connector for µ-Slides (10830, ibidi)
- Hose Clip (10821, part of the ibidi Pump System)
- Sterile 1 ml syringe with simple Luer adapter, biocompatible (various suppliers)
- Standard cell culture equipment (pipettes, tubes, sterile working bench, cell culture incubator, culture flasks, hemocytometer, etc.)
- Inverted microscope

2 Cell Preparation Before Illumination

Perform all steps under sterile conditions.

Important Note for Cell Preparation

The quality of photo-induced cell migration depends heavily on the confluent cell layer and the cells' initial state. For better results with ibiTreat µ-Slides, refer to Application Note 03: Cell Culture in ibidi Channel Slides Using the µ-Slide VI $^{0.4}$ as an Example and Application Note 08: Coating Protocols for ibidi Labware.

NIH-3T3 and Rat1 cells

Prepare the cells at a concentration of 6 × 10^5 cells/ml in cell culture medium (DMEM + 10% FCS).

1. Fill each channel of the µ-Slide VI $^{0.4}$ ibiTreat with 30 µl of the cell suspension.
2. Let the cells adhere in the incubator for 1 hour.
3. Fill the reservoirs on both sides with 60 µl of medium.
4. Incubate overnight or until a confluent monolayer forms. If necessary, exchange the medium.

HUVEC

1. Optionally, coat with ECM protein.
2. Prepare the cells at a concentration of 3 × 10^5 cells/ml in cell culture medium (ECGM with supplements).
3. Fill each channel of the µ-Slide VI $^{0.4}$ ibiTreat with 30 µl of the cell suspension.
4. Let the cells adhere in the incubator for 1 hour.
5. Fill the reservoirs on both sides with 60 µl of medium.
6. Incubate overnight or until a confluent monolayer forms. If necessary, exchange the medium.

3 Preparation of the Phototoxin Solution

Important Note for Phototoxins

In addition to LAP and Irgacure 2959, other phototoxins that react to UV light at 365 nm can be used.

Dissolve Irgacure in DMSO before diluting it in the medium. The total concentration of DMSO should not exceed 5%, as higher concentrations may interfere with the assay due to DMSO toxicity.

Before performing the actual experiment, it is important to determine the optimal concentration of each phototoxin for the specific cell line and experimental setup through preliminary tests.

LAP

1. Prepare a 20 mM LAP solution with ultrapure water.
2. Dilute the LAP in cell culture medium to a final concentration of **6 mM**.

Irgacure

1. Prepare the Irgacure stock solution in DMSO just before use.
2. Dilute the Irgacure in cell culture medium to a final concentration of **16 mM**.

4 Illumination

Important Note for Illumination

Perform all steps under sterile conditions. Ensure sterility during transport between the ibidi Micro Illumination System and laminar flow hood, as well as during the illumination process.

Before conducting the actual experiment, define the optimized illumination dose for each cell line and experimental setting.

The minimum pattern size should be 100 µm, as smaller patterns may produce effects that are difficult to detect.

1. Use the confluent cells from Section 2.
2. Protect the slide from light while replacing the cell culture medium with the phototoxin solution prepared in Section 3. For the µ-Slide VI $^{0.4}$ ibiTreat, wash twice with 100 µl of phototoxin solution (per channel) and add 100 µl of phototoxin per channel.

Please Note: Refer to the respective Instructions for the channel volumes of other µ-Slide formats.

3. Prepare the ibidi Micro Illumination System with the corresponding photomask.
4. Set the intensity and the illumination time, then start the illumination process.
    - **NIH-3T3/Rat1**: 100% for 3 minutes
    - **HUVEC:** 100% for 1 minute
5. Immediately after the illumination step, wash the channels at least twice with the respective channel volume (here: 100 µl of cell culture medium).
6. For static cultivation (e.g., of NIH-3T3 and Rat1 cells, see Section 5.1), place the cells in the incubator and image at specific time points, or acquire a live-cell time-lapse series with the ibidi Stage Top Incubator on an inverted microscope.
7. For a perfused migration experiment (e.g., using HUVEC, see Section 5.2), connect the µ-Slide to the ibidi Pump System after illumination and follow the ibidi Pump System Instructions.

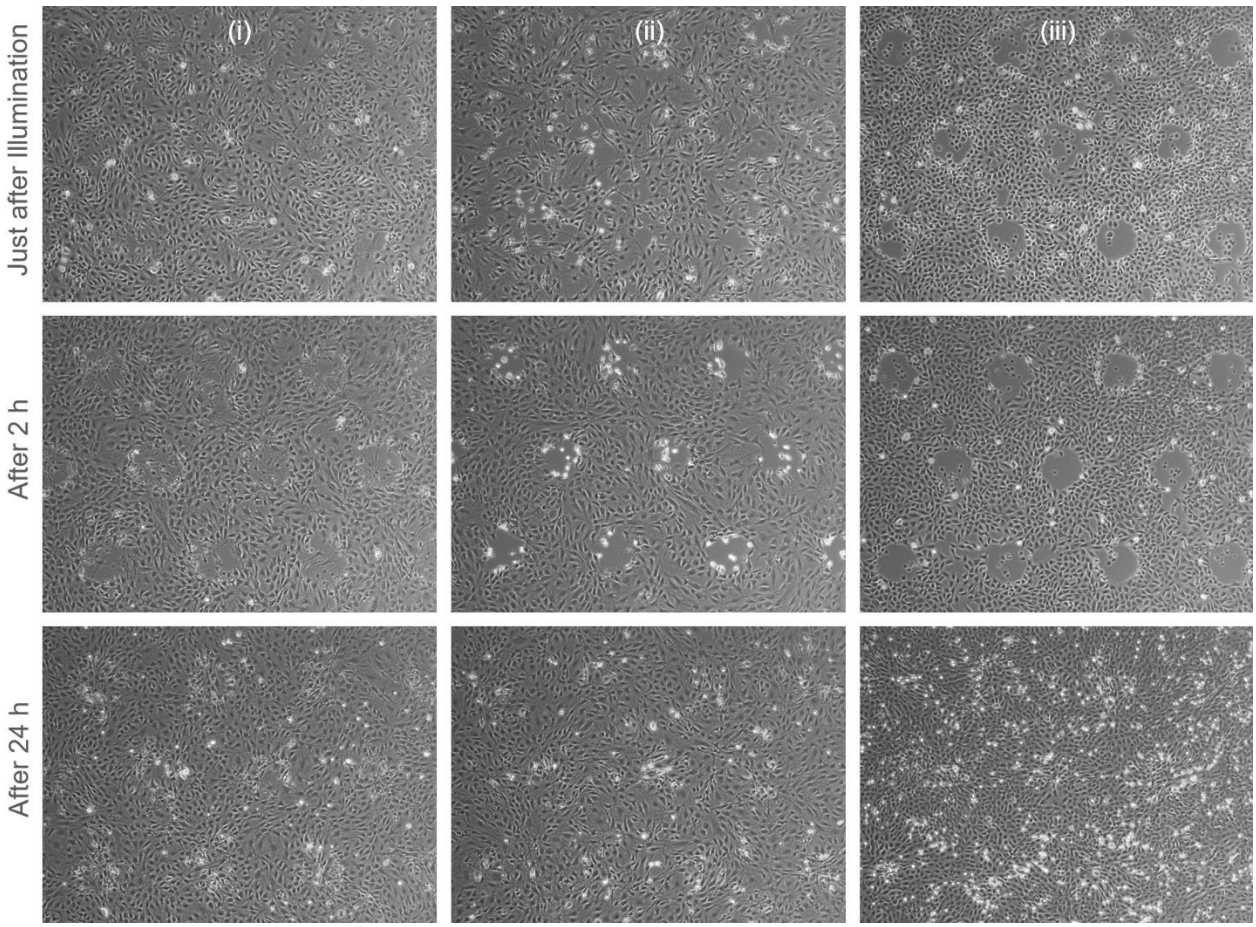
Please Note: The preparation and use of the ibidi Pump System is described in the ibidi Pump System Instructions. Detailed step-by-step instructions for connecting the slide to the perfusion system can be found in Application Note 13: Endothelial Cell Culture Under Perfusion With the ibidi Pump System and the µ-Slide I $^{0.6}$ Luer. Serial connection of µ-Slide VI $^{0.4}$ channels is described in Application Note 31: Serial Connection of µ-Slide VI $^{0.4}$ Channels for Flow Experiments.

5 Results Using Different Experimental Settings

As shown in the experimental examples below, post-illumination cell behavior and viability are significantly influenced by both the cell line and the phototoxin used. The parameters selected for the ibidi Micro Illumination System—such as intensity and illumination duration—along with the phototoxins used in the presented experimental data, serve as initial conditions. These parameters may need to be optimized based on the specific cell line and the desired mode of cell death or cellular response.

5.1 Time-Series Data With Different Phototoxins and Static Cell Cultivation

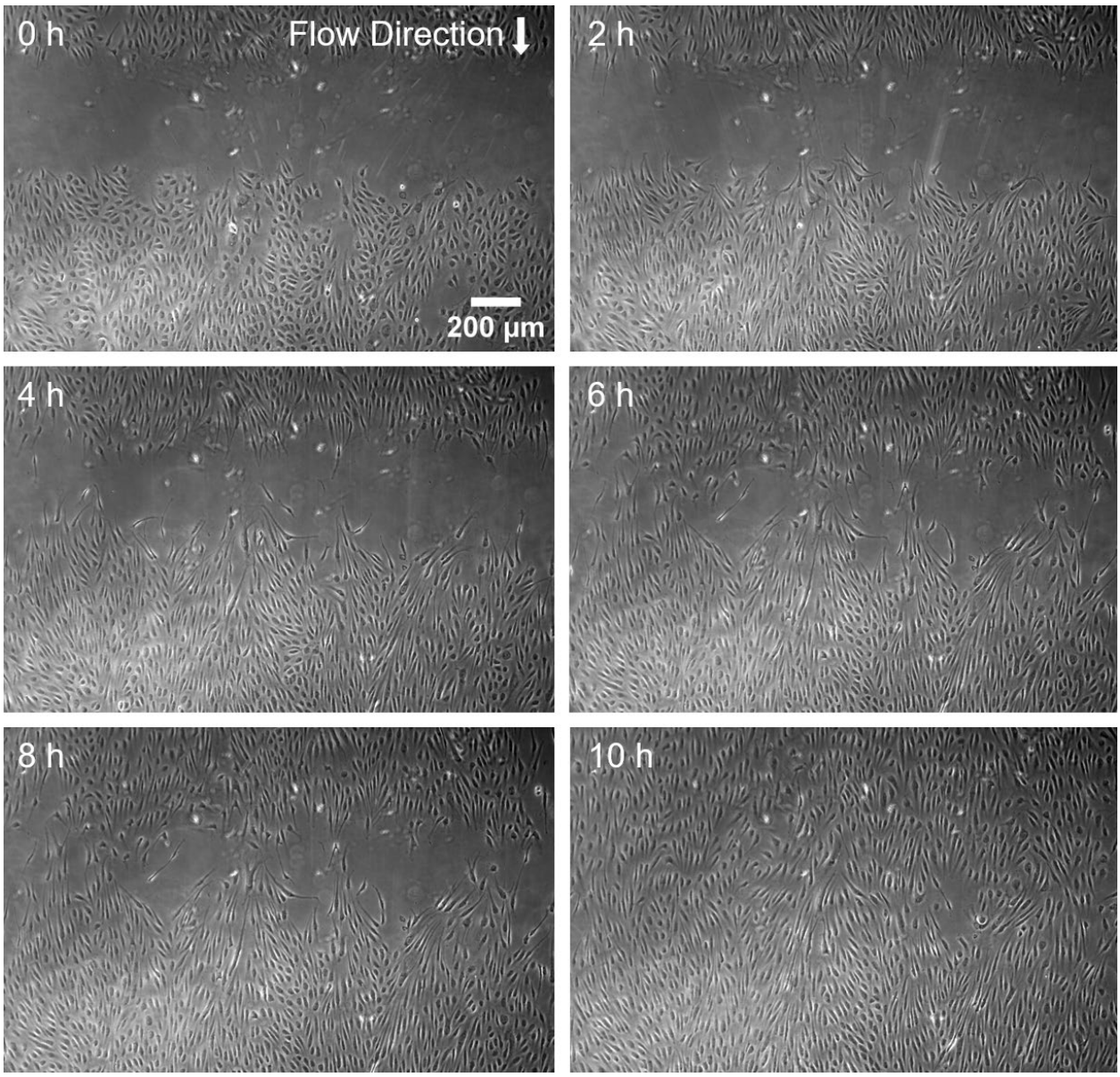
To analyze photo-induced wounding and subsequent cell migration under static conditions, NIH-3T3 and Rat1 cells were cultured in µ-Slide VI $^{0.4}$ ibiTreat and locally illuminated using defined photomask patterns. LAP and Irgacure were applied as phototoxic agents to generate reproducible cell-free regions under standard incubator conditions.



NIH-3T3 cells ((i) and (ii)) as well as Rat1 cells (iii) were cultivated for one day after seeding. Illumination was conducted using a photomask with 200 µm patterns. Either 6 mM LAP ((i) and (iii)) or 16 mM Irgacure (ii) were used as phototoxins. While the patterns were barely visible after illumination in NIH-3T3 cells ((i) and (ii)), they became immediately visible in Rat1 cells (iii). After 2 hours, the patterns became visible under all conditions due to cell death, with variations observed based on the cell line and phototoxin used. Within 24 hours, the cell-free areas were recolonized by cell migration, resulting in the formation of new confluent cell monolayers.

5.2 Time Series of HUVEC Cell Migration With Applied Flow

To assess cell migration under physiologically relevant flow conditions, photo-induced wounding was combined with controlled perfusion in endothelial cells. HUVECs were cultivated in the µ-Slide VI $^{0.4}$ under defined shear stress using the ibidi Pump System prior and after the localized UV illumination. Photo-induced wounding was performed using the photoinitiator LAP as a phototoxic agent and a photomask geometry that allowed the creation of a wide, band-shaped wound. This setup enables the investigation of endothelial migration dynamics in response to photo-induced wounds under continuous flow, thereby mimicking key aspects of vascular environments *in vitro*.



Cell migration of HUVECs under shear stress (10 dyn/cm²) in the µ-Slide VI $^{0.4}$ ibiTreat. HUVECs were cultured on a Matrigel® coating under shear stress for 3 days using the ibidi Pump System before a 450 µm-wide band was optically wounded by UV irradiation. Perfusion was then resumed, and a time-lapse acquisition over 10 hours revealed that cells migrated from both sides to close the gap, thereby restoring a confluent monolayer.