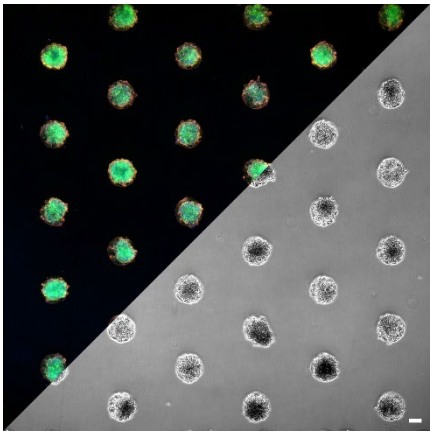
Cell Culture and Immunofluorescence Staining in the µ-Slide VI 0.4 µ-Pattern ibiTreat

This Application Note presents a simple protocol for the cultivation, fixation, and staining of cells or spheroids growing on the µ-Slide VI 0.4 µ-Pattern ibiTreat, cir200, pit600, hex. The ibidi µ-Patterns provide spatially defined adhesive ibiTreat (tissue culture-treated) patterns on the ibidi Polymer Coverslip, surrounded by the Bioinert (ULA) to ensure a 3D cell aggregation only on the defined micropatterned areas.

In this example, human liver cells (Huh7) were cultivated on a multi-cell array and fixed with a formalin solution. The F-actin cytoskeleton, α-tubulin, and nuclei were labeled for fluorescence microscopy.



ibidi Solutions for Micropatterning

- µ-Pattern ibiTreat for Single-Cell Arrays
- µ-Pattern ibiTreat for Multi-Cell Arrays
- µ-Pattern ibiTreat for Line Arrays
- Custom µ-Pattern ibiTreat

Related Documents

- AN 65: Cell Adhesion on ibidi µ-Patterns: Parameters and Optimization (PDF)
- AN 79: Selective and Localized Cell Adhesion using a CD19-Coated Custom µ-Pattern ibiTreat (PDF)
- AN 80: Formation and Long-Term Cultivation of Spheroids in the µ-Slide 8 Well high µ-Pattern ibiTreat (PDF)
- Video: The ibidi µ-Patterning Technology—Achieve Spatially Defined Cell Adhesion with Micropatterning
- Rüdiger, D., *et al.*: Selektive und ortsaufgelöste Zellanbindung mit µ-Pattern ibiTreat, 2023, BIOspektrum, 4, 391

1 Materials

1.1 Reagents and Buffers

- Adherent cells, e.g., Huh-7 (JCRB: #JCRB0403)
- Cell culture medium; e.g., basal medium RPMI-1640 (Gibco, 11875093) with 10 % fetal bovine serum (Gibco, 10270106)
- Phosphate-buffered saline (PBS; 14190144, Gibco)
- Accutase (A11105, Gibco), or other suitable detachment reagent for cell harvesting
- Phosphate-buffered saline (PBS; 14190144, Gibco)
- Formalin, 10 %, ready to use (HT5011, Sigma Aldrich)
- Triton-X-100 (A16046, Thermo Fisher Scientific)
- Permeabilization buffer (0.5 % Triton-X-100 in PBS)
- Bovine serum albumin (BSA) (A1470-10G, Sigma Aldrich)
- Blocking buffer (1 % BSA in PBS)
- Antibody dilution buffer (1% BSA and 0.05 % Triton-X-100 in PBS)
- Phalloidin-iFluor 488 (ab176753, Abcam)
- Monoclonal anti-α-tubulin antibody (T5168, Sigma-Aldrich)
- Anti-mouse-IgG-Atto 594 secondary antibody (76085, Sigma-Aldrich)
- ibidi Mounting Medium With DAPI (50011, ibidi)
- ibidi Immersion Oil 2 (50102, ibidi)

1.2 Equipment

- µ-Slide VI $^{0.4}$ µ-Pattern $^{\text{ibiTreat, cir200, pit600, hex}}$ (83612, ibidi)
- µ-Slide Rack (80003, ibidi)
- Standard cell culture equipment (pipettes, sterile working bench, cell culture incubator, culture flasks, cell culture medium, hemocytometer, etc.)
- Inverted fluorescence microscope with appropriate filter sets

2 Methods

2.1 Cell Seeding and Cultivation

Please read the Instructions before working with the µ-Slide VI $^{0.4}$ µ-Pattern $^{\text{ibiTreat, cir200, pit600, hex}}$. Perform all steps under sterile conditions. It is recommended that the slides and cell culture medium be placed in the incubator a day before seeding the cells to avoid the formation of air bubbles during handling. Before starting the experiment, prepare the Huh-7 cells in a standard cell culture flask (e.g., T75) with adherent cells at the bottom. The cells should ideally be sub-confluent and healthy on the day of the experiment.

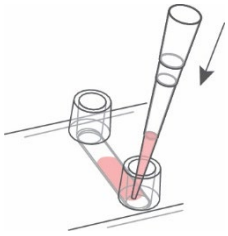
It is essential to work swiftly during the whole procedure to prevent the cells from drying.

If not stated otherwise, all given volumes are per channel, and all incubation steps are at room temperature.

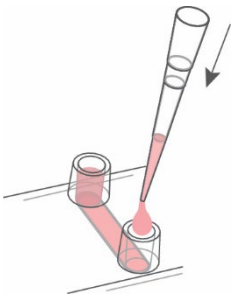
- Add 10 ml Accutase to the T75 flask for cell detachment; incubate for 5 minutes in the incubator (37 °C, 5 % CO_2).
- Harvest the cell suspension, centrifuge, and dilute it in a low amount of cell culture medium for counting.
- Count the cells and adjust to a final concentration of 0.5–3 × 10^6 cells/ml in cell culture medium.

Note: Please optimize the cell seeding concentration depending on the cells used and your experimental needs. For more insight on the parameters that are important for cell adhesion on ibidi µ-Patterns, read the Application Note 65: Cell Adhesion on ibidi µ-Patterns: Parameters and Optimization.

- Unpack a µ-Slide VI $^{0.4}$ µ-Pattern $^{ibiTreat, cir200, pit600, hex}$ and put it on the µ-Slide Rack or an appropriate surface.
- Pipet 30 µl of the cell suspension into each channel by pipetting directly into the channel. Quick dispensing helps avoid trapped air bubbles. Repeat for all channels.
- If necessary, remove trapped air bubbles from the channel by inclining the µ-Slide and tapping on one edge.
- Cover the reservoirs with the supplied lid.
- Put the µ-Slide with the rack into the incubator (37 °C, 5 % CO_2) and let the cells attach for 1 hour.
- Fill 60 µl of cell-free cell culture medium into each of the reservoirs. Do not trap air bubbles.
- Put the µ-Slide with the rack into the incubator (37 °C, 5 % CO_2) and incubate the cells overnight.
- If necessary, wash with cell-free medium to remove non-attached cells and debris the next day.
- For extended cell cultivation, we recommend a continuous medium exchange every 1–2 days (see chapter 2.2). In this example, the cells were cultivated on the patterns for 10 days.



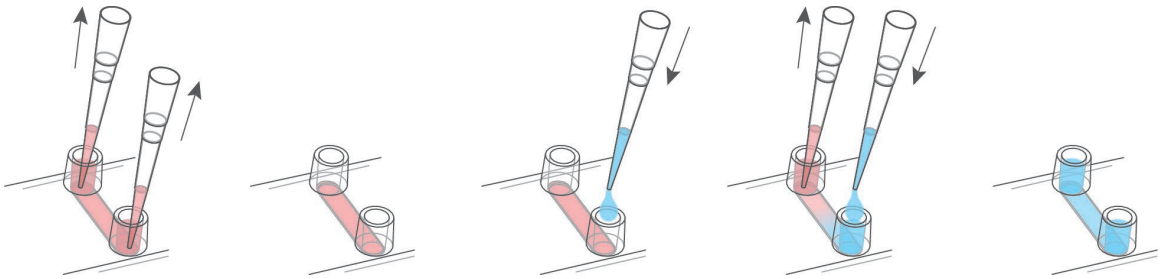
Filling cell suspension into a channel of the µ-Slide VI $^{0.4}$.



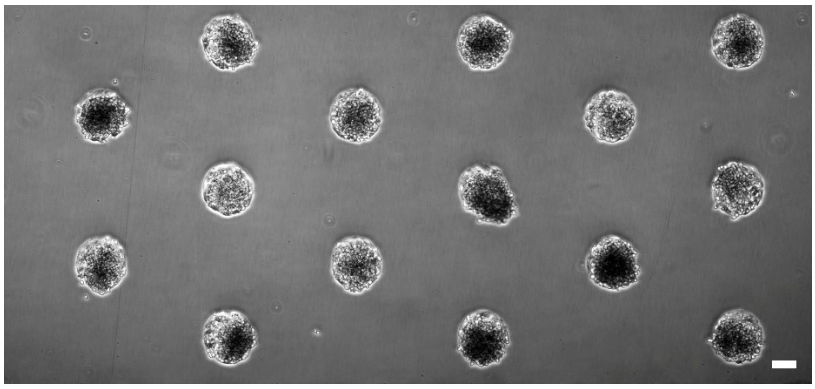
Filling the Luer reservoirs with cell-free culture medium.

2.2 Washing and Continuous Medium Exchange in Channels

- Carefully remove the medium from the reservoirs. Aspirate away from the channel to prevent liquid suction from the channel itself.
- Gently introduce 120 µl of cell-free culture medium into one reservoir, replenishing the channel.
- Aspirate the old culture medium from the opposite reservoir. Be careful when using a cell culture aspiration device, as this may flush away partially attached cells or clusters.
- Refill the reservoirs using 60 µl cell-free culture medium per reservoir.



Continuous medium exchange with a minimum of three times the channel volume.



Phase contrast image of Huh-7 cells cultured for 10 days on ibiTreat µ-Pattern (200 µm circles, 600 µm pitch, hexagonal). Scale bar = 100 µm.

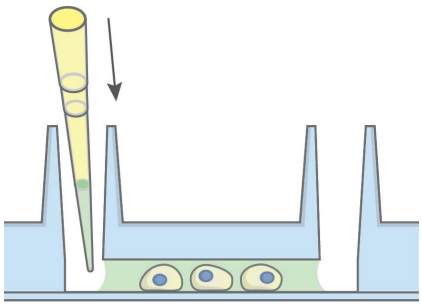
2.3 Fixation, Permeabilization, and Blocking

- Perform all steps quickly to ensure that the channels do not dry out. Aspirate away from the channel to prevent liquid suction from the channel itself. Prepare enough Permeabilization Buffer and Blocking Buffer for the experiment.
- Replace the cell culture medium with PBS through continuous medium exchange for washing (see chapter 2.2)
- Aspirate most of the PBS from the reservoir. Do not aspirate the entire channel volume.
- Fix the cells with 100 µl formalin (10 %) for 20 minutes.
- Wash the cells twice with 200 µl PBS through continuous medium exchange.
- Aspirate the PBS from the reservoirs. Make sure not to aspirate the entire volume of the channel.

- Pipette 100 µl Permeabilization Buffer in one of the reservoirs and incubate the cells for 5 minutes.
- Wash the cells twice with 200 µl PBS through continuous medium exchange.
- Aspirate the PBS from the reservoirs. Make sure not to aspirate the entire volume of the channel.
- Block with 100 µl Blocking Buffer for 20 minutes at room temperature.
- Wash the cells twice with 200 µl PBS as in previous steps.

2.4 Staining

- Prepare a primary staining solution by diluting phalloidin and the anti-α-Tubulin antibody in Antibody Dilution Buffer (both 1:500 dilution, or according to the manufacturer's recommendation).



Note: For the staining procedure, aspirate most of liquid from the reservoir. Do not aspirate the entire channel volume

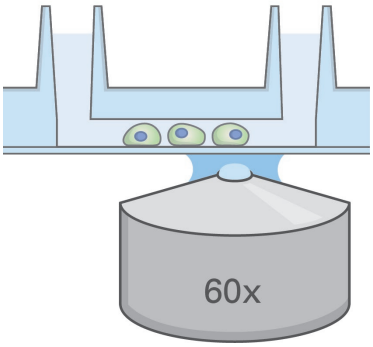
- Exchange the Blocking Buffer in the channels through continuous exchange with 100 µl of the primary staining solution and incubate overnight in the dark.
- Wash twice with PBS as in previous steps.
- Prepare secondary staining solution by diluting the secondary antibody in Antibody Dilution Buffer (1:500, or according to the manufacturer's recommendation).
- Exchange PBS in channels with 100 µl of secondary staining solution and incubate at room temperature for 1 hour in the dark.
- Wash three times with PBS.

2.5 Mounting

- Aspirate all PBS (now, the entire channel volume!) and immediately add ibidi Mounting Medium With DAPI for nuclear staining until the channel is filled. If a different mounting medium is used, please note that it must be non-drying to avoid damage to the µ-Slide.
- Store at 4 °C in the dark until imaging.
- The stained µ-Slide can be stored for up to 4 weeks. Ideally, proceed immediately with imaging since longer storage times can reduce image quality.

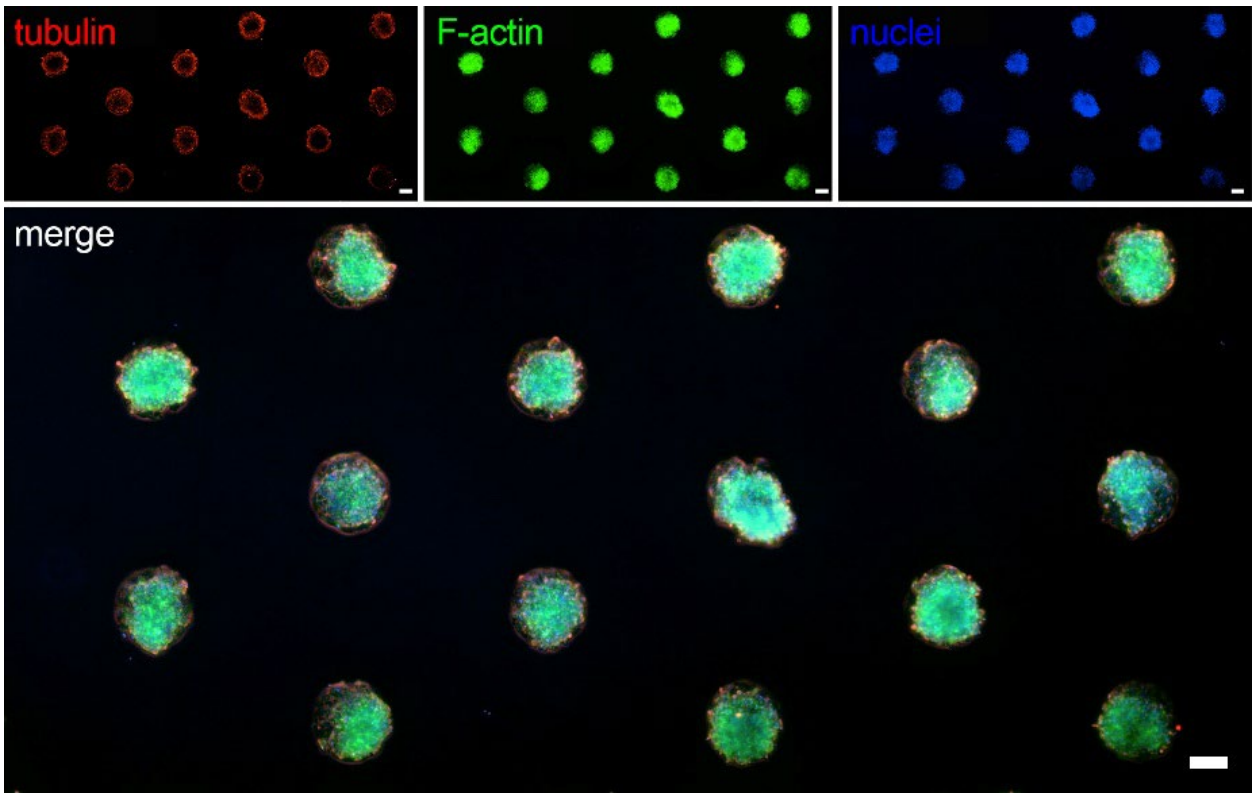
2.6 Imaging

- Observe the cells under a fluorescence microscope with appropriate filter sets and, if necessary, with ibidi Immersion Oil.
- Optionally, overlay channel images to create a merged image.



Note: Find out which immersion oils are compatible with the ibidi labware products at: ibidi.com/oil.

3 Results



Widefield fluorescence microscopy of Huh-7 cells adhered on an ibiTreat µ-Pattern in the µ-Slide VI $^{0.4}$. The F-actin cytoskeleton was visualized using phalloidin (green). Nuclei were stained blue with DAPI, and tubulin red. Imaging was performed on a Nikon TiE inverted microscope using 4x objective. Scale bars = 100 µm.