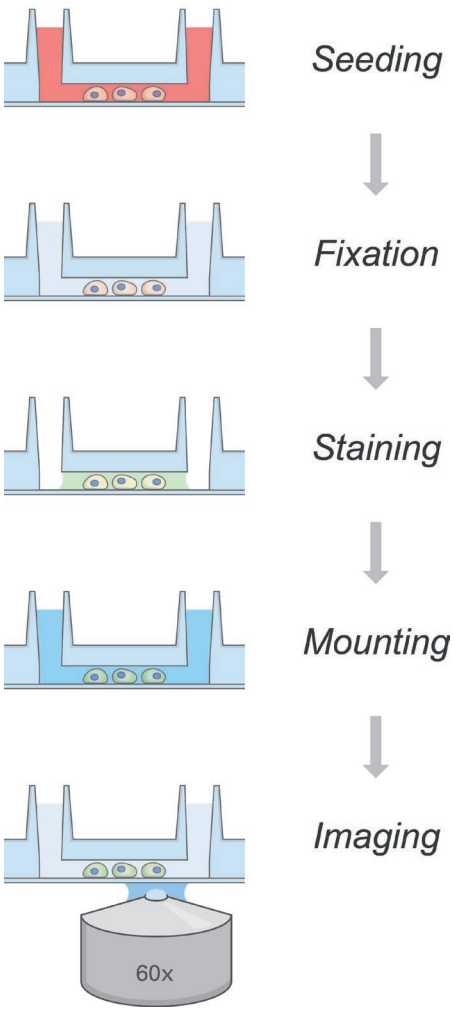
Cell Culture and Fluorescence Staining in the µ-Slide VI 0.4

Fluorescent actin stainings, particularly using phalloidin, are a powerful approach for getting insight into cellular structures and processes using microscopy. This approach allows the specific visualization of actin filaments, making fluorescent actin staining an indispensable tool for scientists to answer numerous questions related to cell biology, including cytoskeletal organization and dynamics.

In this Application Note, we present a simple protocol for the cultivation and fluorescent actin staining of the adherent human fibrosarcoma cell line HT-1080 using the µ-Slide VI 0.4.



For more insights on the benefits of using the µ-Slide VI 0.4, take a look at Application Note 03 “Cell Culture in ibidi Channel Slides”.

Please note: before starting a fluorescent actin staining, several important parameters must be checked, such as the optimal cell density, the ideal cell culture vessel geometry and substrate/coating. In addition, positive and negative controls are necessary to validate the staining. Therefore, we strongly recommend a thorough literature research before the experiment.

Do you want to combine this protocol with an immunofluorescence assay? Find more detailed information about planning and conducting immunofluorescence assays in our Application Chapter “Immunofluorescence”.

1. Materials

1.1. Reagents and Buffers

Cell Culture

- HT-1080 cells (85111505, Sigma Aldrich)
- Cell culture medium: DMEM (D6546, Sigma Aldrich) with 10 % Fetal Bovine Serum (F1283, Sigma Aldrich)
- D-PBS (14190144, Gibco)
- Accutase (A1110501, Gibco)

Fluorescence Staining and Imaging

- D-PBS (14190144, Gibco)
- Formalin, 10 %, ready to use (HT5011, Sigma Aldrich)
- Triton-X-100 (A16046, Thermo Fisher Scientific)
- Permeabilization Buffer (0.1 % Triton-X-100 in D-PBS)
- Bovine Serum Albumin (BSA) (A1470-10G, Sigma Aldrich)
- Blocking Buffer (1 % BSA in D-PBS)
- Phalloidin solution: 1 µl of Phalloidin-iFluor 488 Reagent (ab176753, Abcam) in 1 ml of blocking buffer
- ibidi Mounting Medium With DAPI (50011, ibidi)
- ibidi Immersion Oil (50101, ibidi)

1.2. Equipment

- µ-Slide VI 0.4 ibiTreat (80606, 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

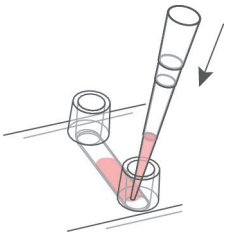
2.1. Cell Cultivation

Please read the Instructions before working with the µ-Slide VI 0.4. Perform all steps under sterile conditions. It is recommended to place the µ-Slide VI 0.4 and the cell culture medium into the incubator the day before seeding the cells to avoid the formation of air bubbles during handling. Before starting the experiment, prepare the HT-1080 cells in a standard cell culture flask (e.g., T75) with adherent cells at the bottom. The cells should ideally be subconfluent and healthy on the day of the experiment.

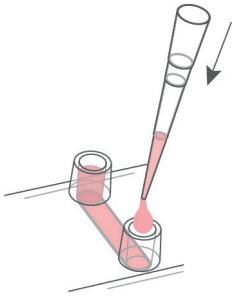
It is essential to work swiftly during the whole procedure to prevent the cells from drying.

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 min 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 3×10^5 cells/ml in cell culture medium.
- Unpack an ibidi µ-Slide VI $^{0.4}$ and put it on a µ-Slide Rack or an appropriate surface.
- Pipet 30 µl of the HT-1080 cell suspension into each channel by pipetting directly into the channel. Quick dispensing helps to avoid trapped air bubbles.
- 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 h.
- 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.
- For extended cell cultivation, we recommend a continuous medium exchange every second day (see Chapter 2.2).



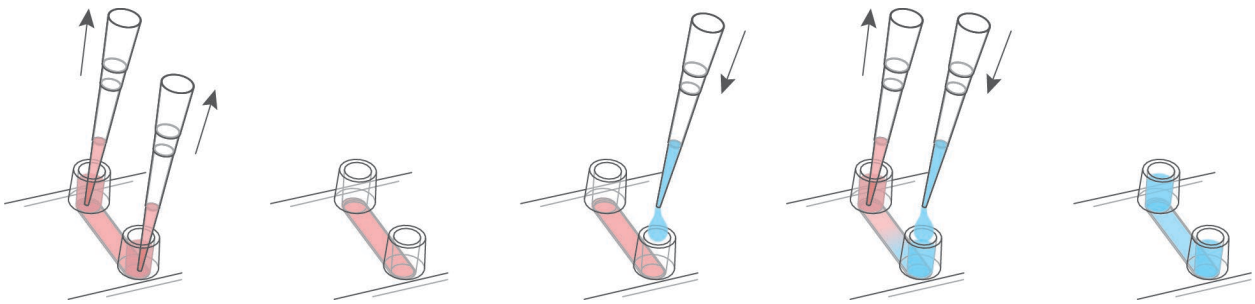
Filling a cell suspension into a channel of the µ-Slide VI $^{0.4}$.



Filling the Luer reservoirs with cell-free culture medium.

2.2. Continuous Medium Exchange

- Carefully aspirate the medium from the reservoirs. Do not aspirate any liquid from the channel itself.
- Gently introduce 120 µl of cell-free culture medium into one reservoir, allowing the channel to replenish.
- 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.



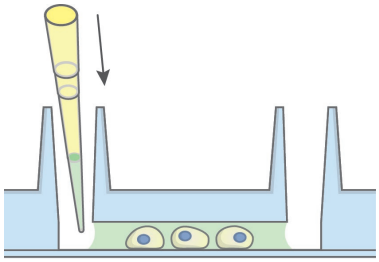
Continuous medium exchange with minimum three times the channel volume.

2.3. Fixation, Permeabilization, and Blocking

- Perform all steps quickly to ensure that the channels do not dry out. Prepare enough Permeabilization Buffer and Blocking Buffer for your experiment.
- Replace the cell culture medium with D-PBS through continuous medium exchange for washing.
- Aspirate most of the D-PBS. Do not aspirate the entire volume.
- Fix the cells with 100 µl formalin (10 %) for 20 min.
- Wash the cells three times with 200 µl D-PBS through continuous medium exchange.
- Aspirate most of the D-PBS. Do not aspirate the entire volume.
- Incubate the cells in 100 µl Permeabilization Buffer for 5 min.
- Wash the cells with 200 µl D-PBS through continuous medium exchange.
- Aspirate most of the D-PBS. Do not aspirate the entire volume.
- Block with 100 µl Blocking Buffer for 20 min.
- Wash the cells with 200 µl D-PBS.

2.4. Staining

- Aspirate all D-PBS from reservoirs and channel. The use of a 5 ml syringe to fill the empty channels minimizes the risk of introducing air bubbles.
- Immediately apply 30 µl of the phalloidin solution to the channel; incubate for 20 min in the dark. From this point on, the samples should be kept in the dark whenever possible.
- Wash the cells twice with 200 µl D-PBS through continuous medium exchange.

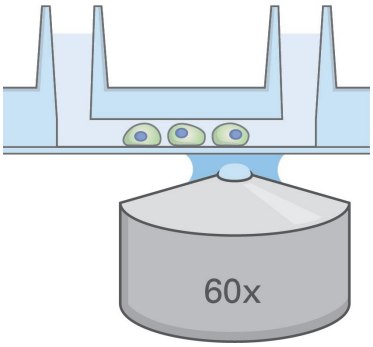


2.5. Mounting

- Aspirate all D-PBS 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 of 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.

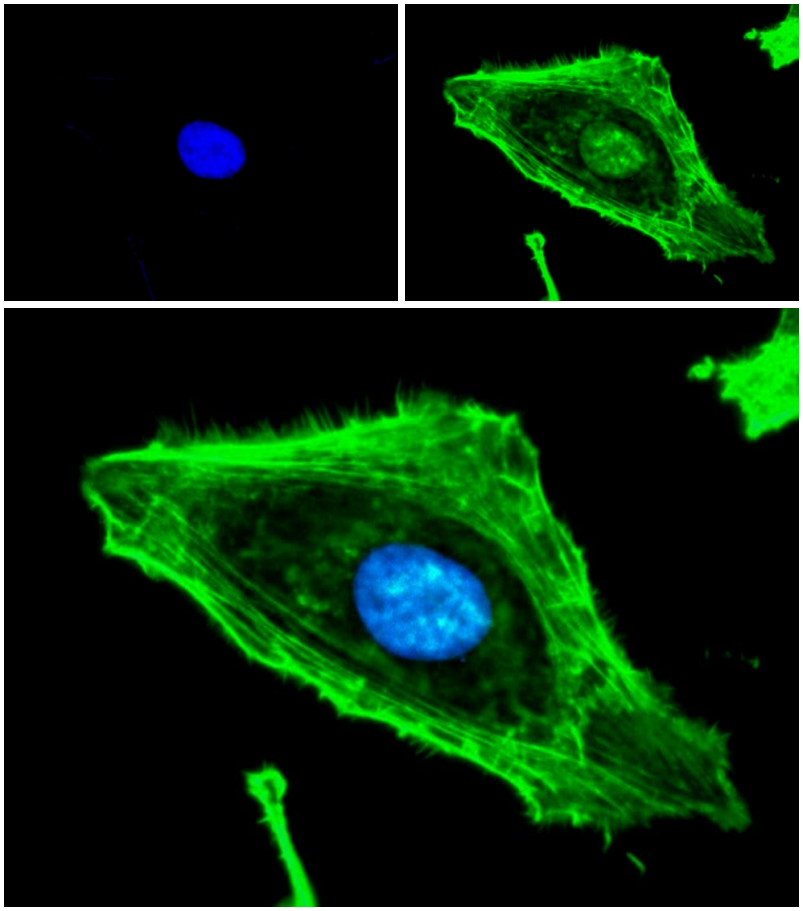
2.6. Imaging

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



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

3. Results



Widefield fluorescence microscopy of HT1080 cells in a µ-Slide VI [0.4]. The F-actin cytoskeleton was visualized using phalloidin (green). Nuclei were stained with DAPI (blue). The imaging was performed on a Zeiss Axiovert 135 microscope using a Plan-Neofluar 40x/0.75 objective lens.

Your fluorescence staining did not work out as expected? Please refer to our immunofluorescence troubleshooting guide!