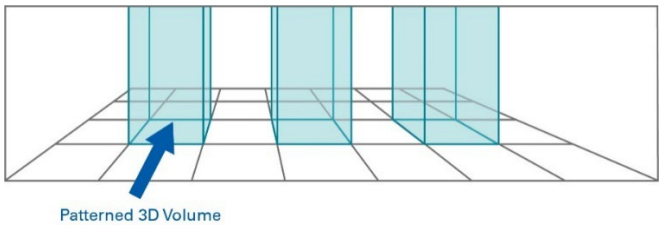
3D Hydrogel Constriction in the µ-Slide I Luer Using the ibidi Micro Illumination System

The ibidi Micro Illumination System enables the spatially controlled structuring of photocrosslinkable hydrogels to generate 3D *in vivo*–inspired barrier assays in microfluidic environments. Localized illumination of the hydrogel induces spatially defined crosslinking, while non-illuminated areas of the hydrogel can be selectively removed. This approach generates 3D-structured hydrogels that form localized flow barriers while still allowing nutrient supply to the cells. The illumination pattern can be tailored by the photomask design, enabling precise control over the geometry of the 3D structuring of the hydrogel.

This Application Note outlines a protocol for customizing a microfluidic channel in ibidi µ-Slides using photocrosslinkable hydrogels. The arteriosclerosis example below describes the generation of a GelMA hydrogel constriction inside the channel of the µ-Slide I $^{0.6}$ Luer in combination with endothelial cell culture and defined perfusion using the ibidi Pump System.



ibidi Solutions for 3D Hydrogel Constriction in the µ-Slide I Luer

- ibidi Micro Illumination System
- µ-Slide I $^{0.6}$ Luer ibiTreat
- ibidi Pump System

Related Documents

- Instructions ibidi Micro Illumination System (PDF)
- Instructions ibidi Pump System (PDF)
- Instructions µ-Slide I Luer (PDF)
- Instructions ibidi Mounting Medium (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 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 75: Structuring a Photoresist-Coated Wafer With Photolithography Using the ibidi Micro Illumination System (PDF)
- Application Note 76: Photo-Induced Cell Migration Using the ibidi Micro Illumination System (PDF)
- Photomask Templates (ZIP)

1 Material

1.1 Reagents and Buffers

- Human umbilical vein endothelial cells (HUVEC, C-12203, PromoCell)
- Endothelial cell growth medium (C-22010, PromoCell)
- Gelatin-methacryloyl (GelMA) (900622, Sigma-Aldrich)
- Lithium-phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) (900889, Sigma-Aldrich)
- PBS (14190144, Gibco)
- Sterile, ultrapure water

1.2 Equipment

- ibidi Micro Illumination System (76000, ibidi)
- 3” photomask (compugraphics, MacDermid Alpha)
- µ-Slide I $^{0.6}$ Luer ibiTreat (80186, ibidi)
- ibidi Pump System (10902, ibidi)
- Perfusion Set RED, 15 cm, ID 1.6 mm, 10 ml Reservoir (10962, ibidi)
- Hose Clip (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, Petri dish, hemocytometer, etc.)
- Inverted microscope

1.3 Optional Cell Staining Material

- PBS (14190144, Gibco)
- Formalin, 10%, ready to use (HT5011, Sigma-Aldrich)
- 4′,6-diamidino-2-phenyl-indole (DAPI) (D9542 Sigma-Aldrich)
- Phalloidin 488 conjugate (ab176753, Abcam)
- VE-cadherin monoclonal antibody (14-1449-82, Invitrogen)
- Anti-mouse IgG Atto 594 (76085, Sigma-Aldrich)
- Triton-X-100 (A16046, Thermo Fisher Scientific)
- Bovine serum albumin (BSA) (A1470-10G, Sigma-Aldrich)
- Blocking buffer (1% BSA + 0.2% Triton X-100 in PBS)
- Permeabilization buffer (0.5% Triton X-100 in PBS)
- Antibody dilution buffer (1% BSA + 0.05% Triton X-100 in PBS)
- ibidi Mounting Medium (50001, ibidi)

2 Hydrogel Preparation and 3D-Structuring by Illumination

2.1 Preparation of the Hydrogel Solution

Additional Information for Hydrogel Solution With GelMA and LAP

Due to the high viscosity of GelMA, it is recommended to use special pipette tips with a large opening. Alternatively, you can use pipettes designed for high viscosity solutions. Among others, we recommend Eppendorf Visco Tips or Gilson Microman E.

After adding LAP, the solution must be protected from light and illumination should be started as soon as possible.

Perform all steps under sterile conditions.

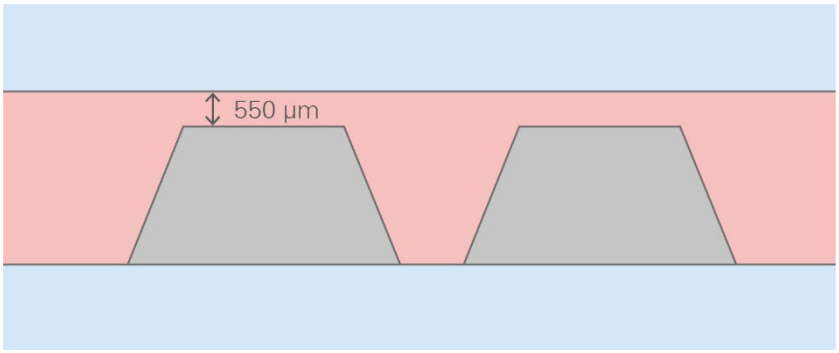
1. Prepare a 20 mM LAP solution with ultrapure water.
2. Prepare a 10% GelMA stock solution according to the manufacturer's instructions.
3. Heat the 10% GelMA to 40°C for at least 30 minutes. If the GelMA is not directly used for illumination, keep at 40°C.
4. Prepare the hydrogel solution containing 5% GelMA and 4 mM LAP, following the pipetting scheme in Table 1. After adding the LAP, keep the solution in the dark and start the illumination as soon as possible.

Hydrogel solution with 5% GelMA and 4 mM LAP	
10% GelMA stock	100 µl
PBS	60 µl
20mM LAP stock	40 µl
Total volume	200 µl

Table 1: Pipetting scheme for a hydrogel solution. All ingredients are listed in the order of pipetting.

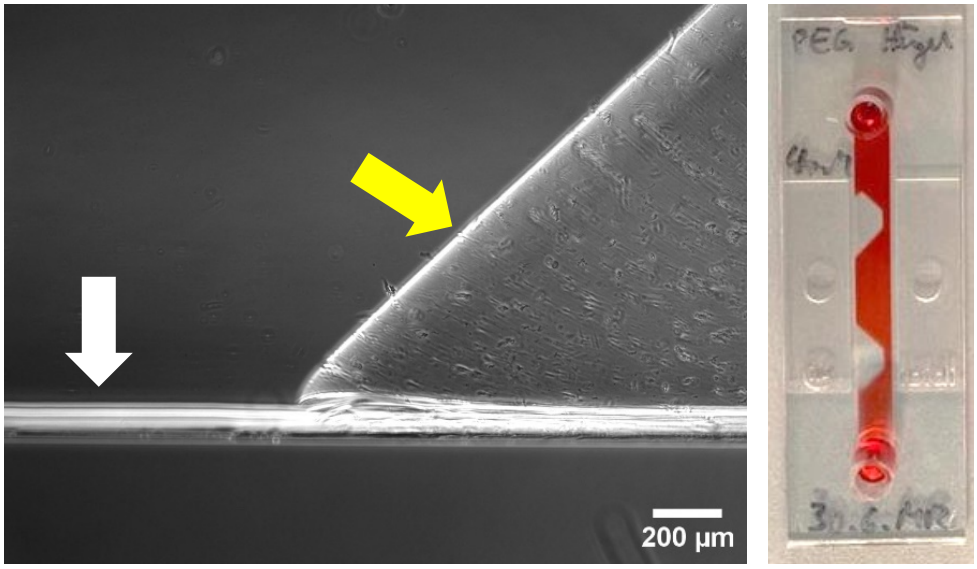
2.2 Illumination

For the arteriosclerosis model shown here, a structure is created that constricts the channel of the µ-Slide I 0.6 Luer at two points but does not completely close it. Please read the Instructions before working with the µ-Slide I Luer.



Schematic drawing of a top view of the constriction geometry of the GelMA hydrogel (grey) in the µ-Slide I 0.6 Luer channel, filled with medium (red).

1. Fill the channel of the µ-Slide I 0.6 Luer with 180 µl of the hydrogel solution prepared in 2.1.
2. Put the photomask and the slide into the ibidi Micro Illumination System. Following the Instructions of the ibidi Micro Illumination System, illuminate for **20 seconds with 30% intensity.** Please find the photomask template used for this Application Note here.
3. After illumination, wash the channel 4 times with 1 ml PBS as soon as possible. Use a syringe and wash twice from each side of the channel.
4. Optionally, check the quality of the hydrogel under a microscope. Clear edges should be visible, and no hydrogel solution residues should be floating in the channel, as shown in the image below.



Left: Microscopy image of the GelMA hydrogel after washing the channel. The image shows a close-up of the gel (yellow arrow) close to the channel wall (white arrow). Right: photo of the whole µ-Slide I 0.6 Luer. Filling the channel with red food coloring highlights the GelMA structure.

3 Cell Seeding and Perfused Cultivation of Endothelial Cells

Perform all steps under sterile conditions. Before starting the experiment, prepare the HUVECs in a standard cell culture flask with the adherent cells at the bottom. The cells should be healthy and optimally subconfluent on the day of the experiment. If not stated otherwise, all incubation steps are at room temperature.

3.1 Cell Seeding

1. Treat the HUVECs with Accutase for 1–2 minutes for detachment.
2. Harvest the cell suspension, centrifuge, and dilute it in endothelial cell growth medium (ECGM) for counting.
3. Count the cells and adjust them to the final concentration of 5×10^5 cells/ml in ECGM.
4. Wash the channel twice with 200 µl ECGM.
5. Pipette 200 µl of cell suspension into the channel (excess fluid will fill the Luer adapters).
6. Remove the leftover cell suspension from the Luer adapters with a standard pipette tip and cover the Luer adapters with the supplied caps to maintain sterility.
7. Put the slide and a wet tissue in a Petri dish and place in the cell culture incubator (37°C, 5% CO_2) for 2 hours for cell adhesion.
8. Wash the channel with 200 µl ECGM to remove non-adherent cells.
9. Fill both Luer ports with 60 µl ECGM, cover the Luer adapters with the supplied caps to maintain sterility, and put the slide with a wet tissue in a Petri dish back in the cell culture incubator (37°C, 5% CO_2) for 24 hours.

3.2 Connecting to the ibidi Pump System for Perfusion

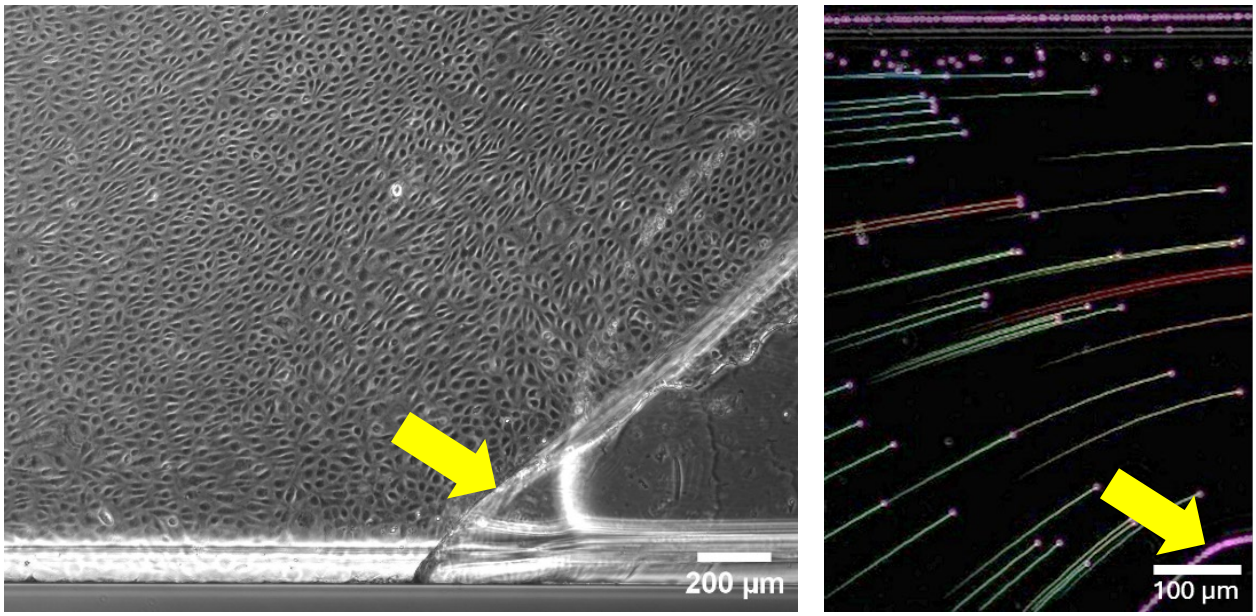
1. Prepare the ibidi Pump System as described in the ibidi Pump System Instructions and fill the ECGM into the reservoirs.
2. After 24 hours cell adhesion, connect the slide to the perfusion system filled with the ECGM. Detailed step-by-step instructions are provided in Application Note 13: Endothelial Cell Culture Under Perfusion With the ibidi Pump System and the µ-Slide I $^{0.6}$ Luer.

4 Optional Cell Staining

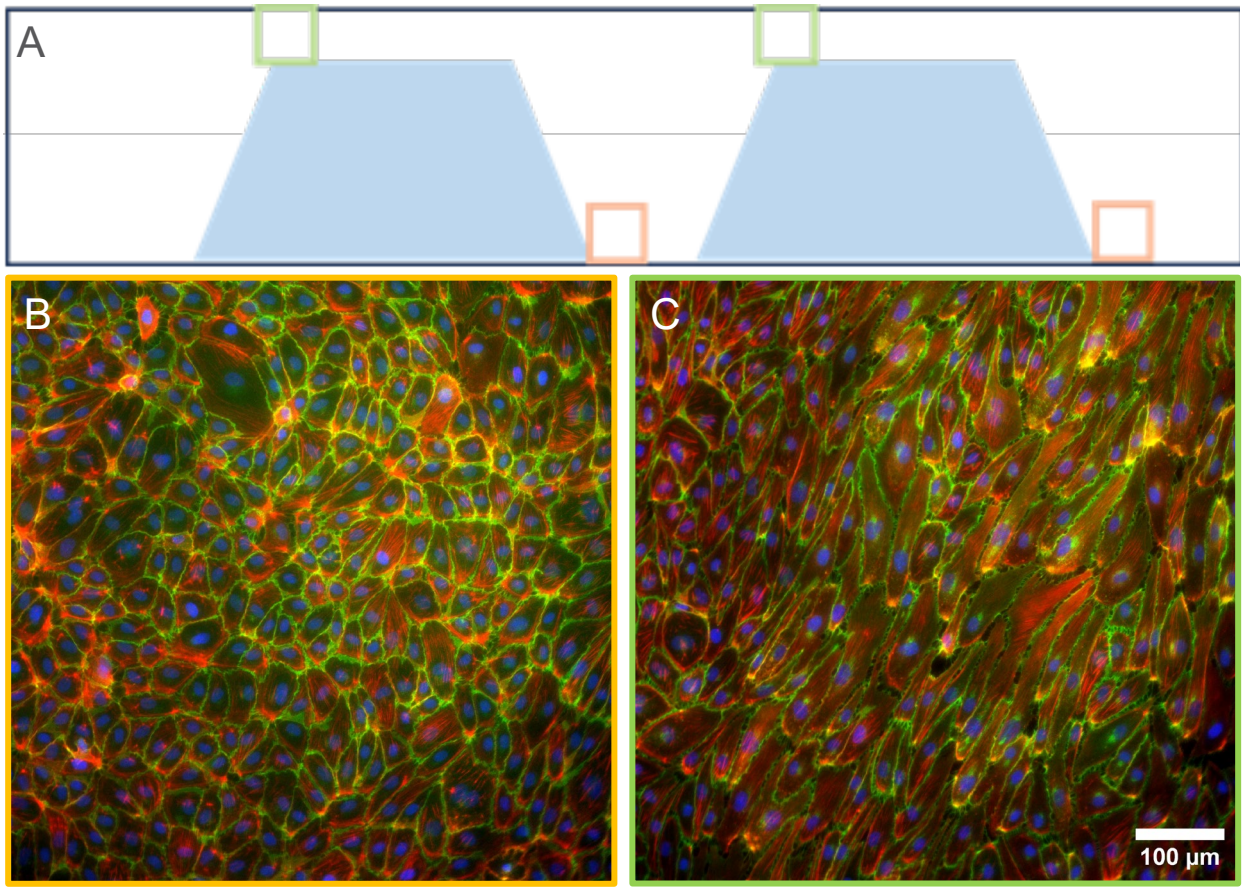
1. Prepare enough permeabilization buffer, blocking buffer and antibody dilution buffer for your experiment according to the material list 1.3.
2. Disconnect the slide from the pump.
3. Aspirate the remaining cell culture medium from the Luer port using a pipette.
4. Gently wash the cells twice with 200 µl PBS by filling PBS in one Luer port until it exits the opposite Luer port, then remove the liquid from the opposite port.
5. To fix the cells, add 200 µl 10% formalin to the Luer port. Make sure the formalin runs through the entire channel and then remove it from the opposite Luer port. For fixation, add an additional 200 µL of formalin to the Luer port and incubate the cells for 10 minutes at room temperature.
6. Remove the formalin and wash the cells three times with 200 µl PBS.
7. Incubate the cells in 200 µl permeabilization buffer for 15 minutes (exchange the PBS with the permeabilization buffer, as described in Step 5).
8. Remove the permeabilization buffer and wash the cells twice with 200 µl PBS.
9. Block with 200 µl blocking buffer for 30 minutes (exchange the PBS with the blocking buffer, as described in Step 5).
10. Dilute the VE-cadherin monoclonal antibody (or other primary antibody) in antibody dilution buffer (1:50 dilution).
11. Exchange the blocking buffer with 200 µl of the primary antibody staining solution, as described in Step 5, and incubate the cells for 2 hours at room temperature.
12. In all following steps, the samples should be kept in the dark whenever possible to avoid photobleaching effects.
13. Wash three times with 200 µl blocking buffer.
14. Prepare the secondary antibody staining solution by diluting anti-mouse IgG Atto 594 (secondary antibody, 1:200), phalloidin (1:1000), and DAPI (final concentration of 1 µg/ml) in antibody dilution buffer.
15. Exchange the blocking buffer with 200 µl of the secondary antibody staining solution, as described in Step 5 and incubate for 1 hour in the dark at room temperature.
16. Wash three times with 200 µl blocking buffer.
17. Exchange the blocking buffer with 200 µl PBS, as described in Step 5.
18. Remove the PBS from the channel. To inject the ibidi Mounting Medium into the channel, press the dropper bottle tightly onto the Luer port. It is recommended to fill the entire channel.
19. Store the slide at 4°C in the dark until imaging.

5 Results

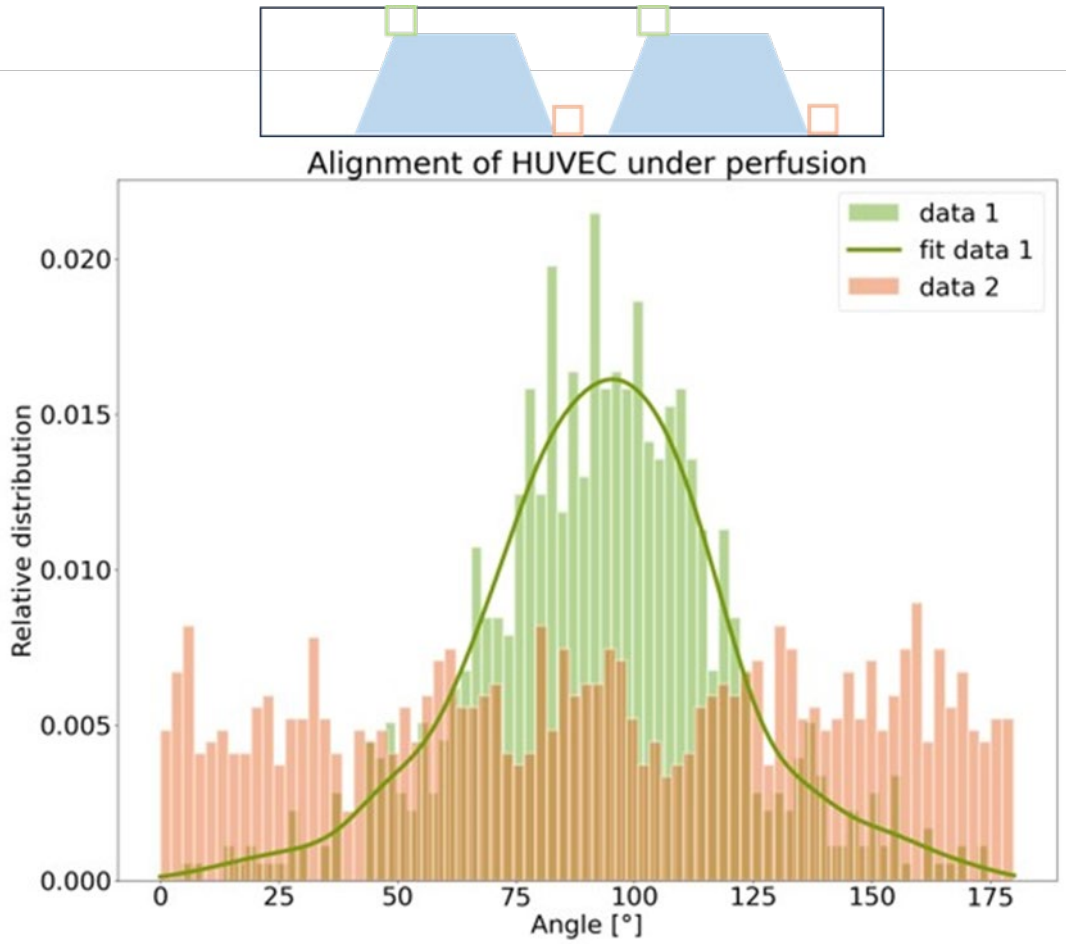
Microscopy images show the polymerized hydrogel barrier and the morphology of the cells. To indirectly visualize the flow distribution caused by the hydrogel bifurcations, the trajectories of fluorescent beads were tracked and color-coded according to their speed as they moved within the channels. Immunofluorescent staining of cell-cell contacts can also be used for quantitative analysis of cell morphology in relation to the flow direction.



Left: phase contrast image of endothelial cells (HUVECs) in the µ-Slide I 0.6 Luer with a 3D-structured GelMA hydrogel barrier (yellow arrow) and a Matrigel® coating after 5 days of cultivation. The example image shows different, region-specific morphological characteristics of the cells, due to the spatial differences in flow velocity. Right: for visualization of the flow inside the µ-Slide I 0.6 Luer with a 3D structured GelMA hydrogel (indicated by yellow arrow), a solution with fluorescent beads (10 µm beads in PBS) was filled in the channel and perfusion was applied. The trajectories of the beads can be tracked and displayed using the ImageJ plugin TrackMate. The speed was then converted into a color code, from slow (blue) to fast (red). The beads are accelerated towards constriction (shift from blue to red), which illustrates the changed flow profile in the channel. This highlights the physiological relevance as an arteriosclerosis model.



Staining of endothelial cells (HUVECs) after 5 days of cultivation in the µ-Slide I 0.6 Luer with GelMA. Nuclei: DAPI (blue), actin filaments: phalloidin 488 conjugate (red), VE-cadherin: Atto 594 (green). A) Schematic top view drawing of the 3D GelMA hydrogel barriers in the channel. The two exemplary green boxes indicate constricted areas, and the orange boxes indicate areas with less flow but higher turbulence. (B) Cells imaged in the orange boxes are more densely packed and smaller in size due to lower flow. (C) Cells imaged in the green boxes are more elongated due to the higher linear flow.



The VE-cadherin signal shows cell outlines, which can be used to determine cell orientation relative to the flow. The VE-cadherin staining images were analyzed in ImageJ, each cell was fitted as an ellipse via a threshold and the "Analyze Particles" evaluation. This can be used to determine the orientation of the cells (angle to the y-axis). The angular distribution was plotted in a histogram. The flow direction corresponds to a 90° angle. The cells in the green constricted areas show a clear preference for the flow direction (histogram peak at around 90°), while the cells in the orange areas of the GelMA show no preferred direction and are evenly distributed over all angles. This highlights the effect of the constriction in combination with perfusion on cell orientation, cell morphology, and cell behavior.