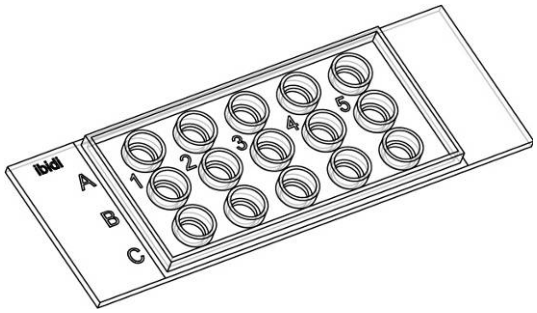
Tube formation assays in µ-Slide Angiogenesis

1. General Information

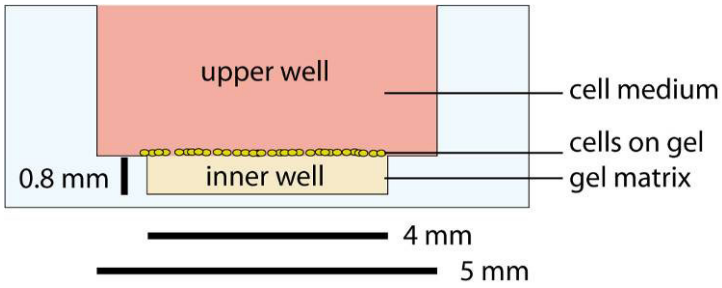
The µ-Slide Angiogenesis is designed for tube formation observation on an inverse microscope. It can be used with all common gel matrices like Matrigel®, collagen gels and hyaluronic acid gels. Only 10 µl gel per well are needed.

The platform provided by µ-Slide Angiogenesis eliminates the meniscus effect often observed in other well formats. Every cell on the flat gel surface is observable with high quality phase contrast or fluorescence microscopy.

For easy handling the wells are compatible with multi-channel pipettes. The plastic is compatible with various fixing solutions such as isopropanol, methanol, paraformaldehyde and others. The optical properties of the plastic bottom are comparable to those of glass coverslips.



µ-Slide Angiogenesis



Cross section of one well

This application note describes an example setup with µ-Slide Angiogenesis for tube formation with endothelial cells (HUVEC) on Matrigel®.

2. Material:

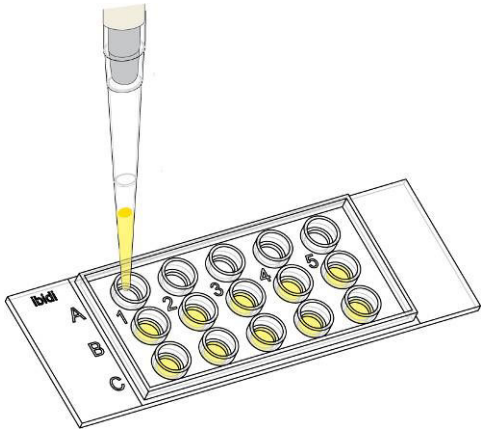
Cells:	HUVEC (Lonza)	10^4 per well
Medium:	Endothelial Cell Growth Medium (Lonza)	50 µl per well
Gel Matrix:	Matrigel® (BD)	10 µl per well
Slides:	µ-Slide Angiogenesis, ibiTreat (ibidi)	1 Slide
Fluorescence stain:	Calcein AM (Lonza)	1 ml (6.25 µg/ml)
Other:	Scale paper for checking	1 sheet

3. Preparation of the gel and the slide:

- The day before seeding cells place the Matrigel® at 4°C on ice in the refrigerator. The gel can slowly thaw over night.

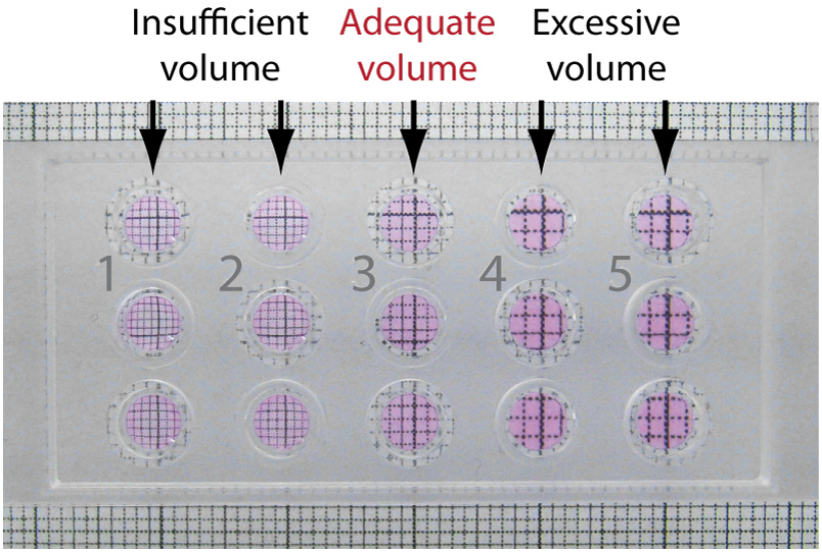
Note: Always use precooled pipet tips (4°C) for pipetting the gel!

- When starting the experiment place the vessel with the gel on ice in the laminar flow.
- Remove the µ-Slide Angiogenesis from the sterile packing and place it on a µ-Slide Rack.
- Apply 10 µl of gel per inner well. Due to the viscosity of the gel it might be useful to set the pipet on some extra volume (e.g. 11 µl). Try this in a preliminary test see below).
- Place the slide in the incubator and let the gel polymerize for 30 minutes.



Pipetting the gel into the inner wells.

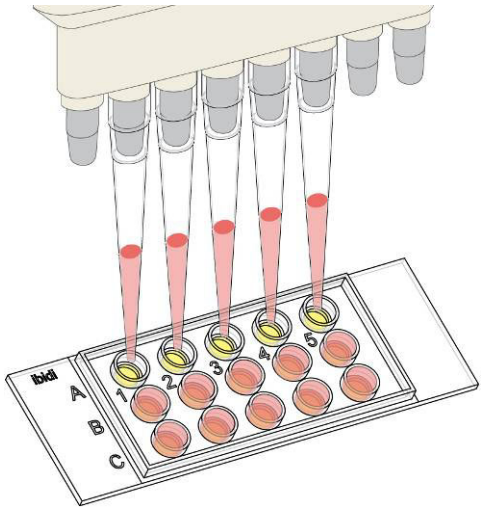
Note: How to adjust the right volume of gel:
The volume in the inner well is adjusted to 10 µl when no magnification or demagnification effect is observed as shown in the picture below.
Try different pipetting volumes and check with a scale paper which setting is adequate. For visualizing the effect hold the slide in a distance of some centimeters over a scale paper.



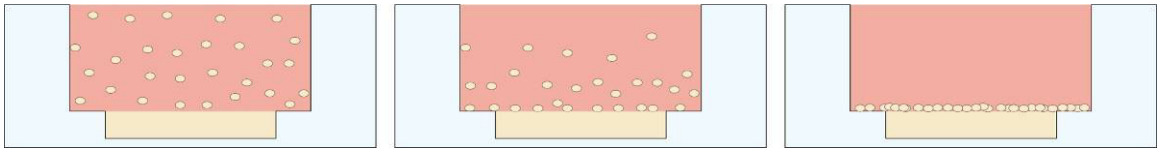
µ-Slide Angiogenesis filled with Matrigel. Columns 1 and 2 contain less than 10 µl. The grid looks diminished. Column 3 is filled with the adequate volume of 10 µl and shows no shift. Columns 4 and 5 have an excessive volume. The grid is magnified.

4. Seeding cells

- For a final cell number of 10.000 cells per well adjust a cell suspension of $2 \cdot 10^5$ cells/ml.
- Apply 50 µl cell suspension to each well. For this step a multi-channel pipet might be helpful.
- Close the slide with the lid and place it in the incubation stage on the microscope for observation.
- After some minutes all the cells have sunk to the ground and are laying in one level. Due to the geometry of the slide the cells on the margins are placed on the plastic surface.

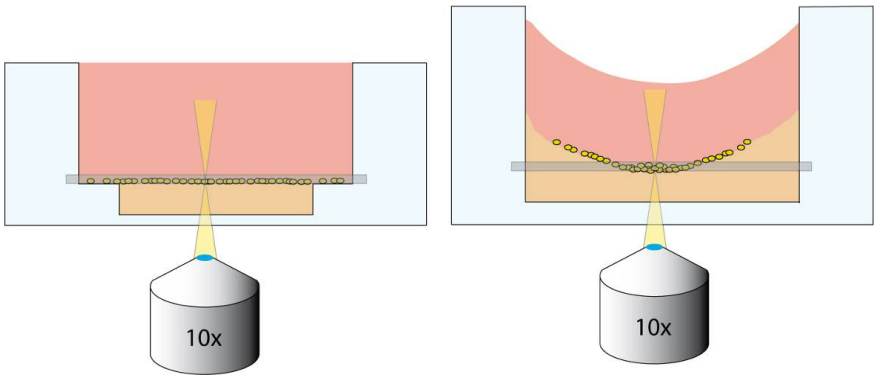


Filling the cell suspension with a multi-channel pipet into the outer wells.



Sinking process of cells. After some minutes all of the cells have fallen to the ground.

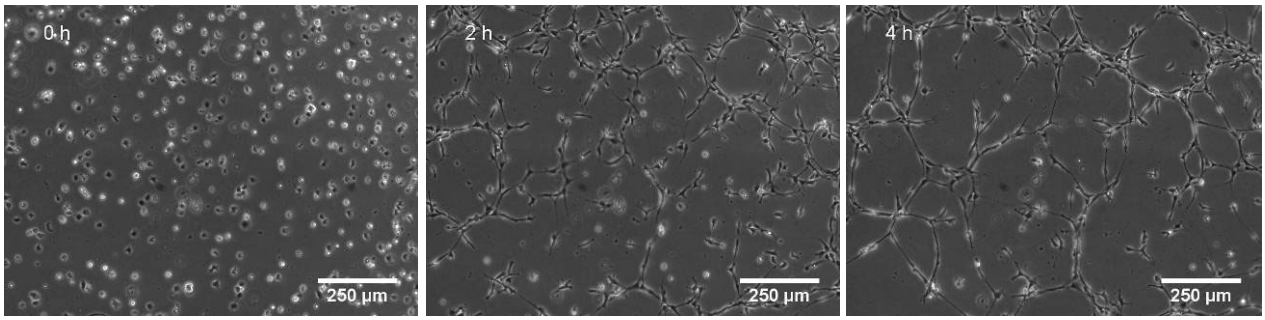
The cross section of the well shows now two flat surfaces: the Matrigel® itself and the medium above. In comparison to normal well formats no meniscus is disturbing the excellent optical properties.



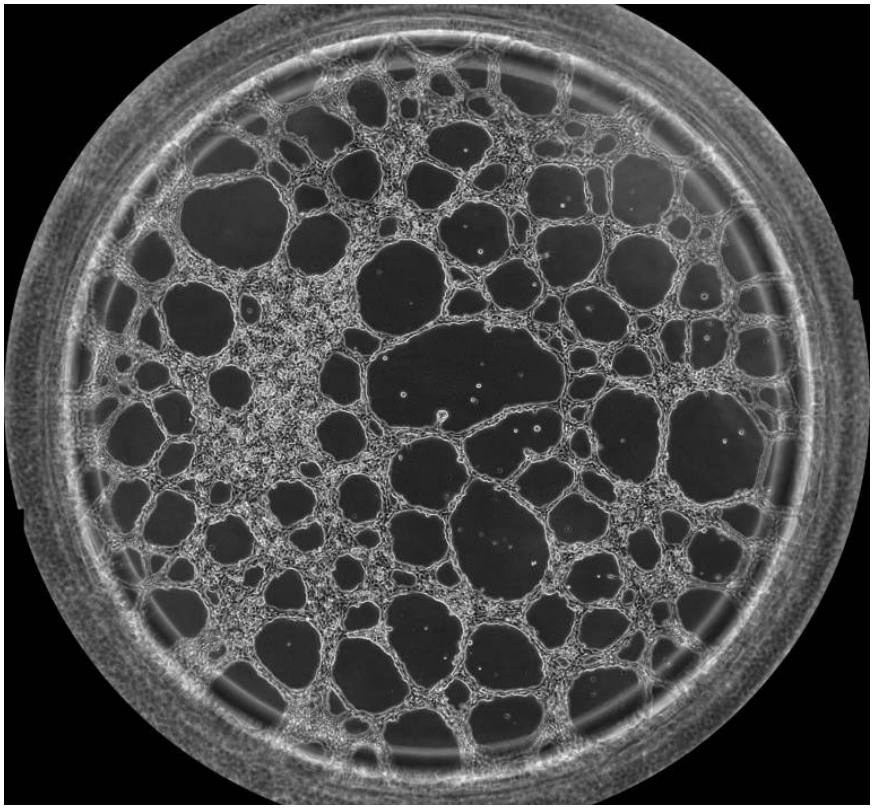
Comparison between the flat surface of a well in µ-Slide Angiogenesis and a well of a 96-well plate.

5. Observation on the microscope

Immediately after seeding the cells position the slide on an inverted microscope with an incubation chamber (e.g. ibidi heating system). The cells are now falling down and starting to build a network of connected cells.
Choose the section you want to observe on your imaging system and start a time lapse recording. For HUVEC we recommend a small magnification and a time interval between the single images of 5 minutes.



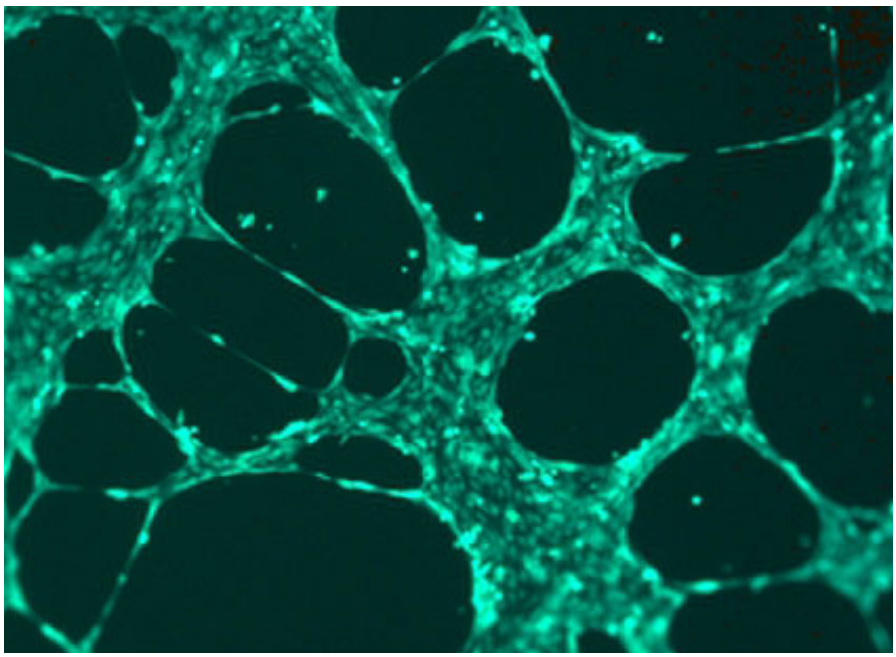
Time Lapse pictures with a 10x magnification at several points of time (0h, 2h, and 4h)



Phase contrast picture with a 10x objective (arranged of 5x6 single pictures) showing a whole well with HUVEC forming a cell network. The image was taken 10 hours after seeding.
The diameter of the well is 4 mm.

6. Staining Protocol

- Photograph the wells before staining to have a comparison of cell pattern before and after staining.
- Carefully discard the supernatant. Take care not to damage the gel or the cell network.
- Add 50 µl serum free medium with diluted calcein (12,5 µl calcein stock 1 µg/µl) at a final concentration of 6.25 µg/ml (1:160).
- Incubate 30 minutes at room temperature in the dark.
- Wash three times with PBS. Rinse the PBS slowly over the side of the upper well. Don't pipette it directly on the cells. Remove it on the other side of the well so that it rinses the cells very softly.
- Take fluorescence pictures at 485 nm/ 529 nm.



HUVEC network stained with calcein (6.25 µg/ml)