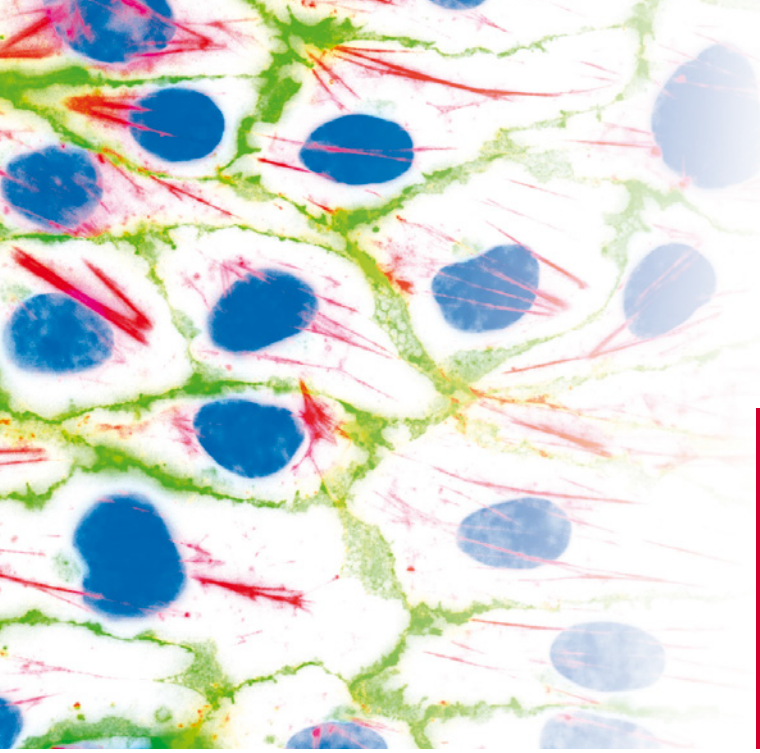


cells in focus ibidi®

ibidi Application Guide

Cell Culture Under Flow

Selected Publications

T. G. Walsh et al. Stabilization of Brain Microvascular Endothelial Barrier Function by Shear Stress Involves VE cadherin Signaling Leading to Modulation of pTyr Occludin Levels. Journal of Cellular Physiology, 2011, 10.1002/jcp.22655

T. Keeley, R. Siow, R. Jacob and G. Mann. A PP2A-mediated feedback mechanism controls Ca2+-dependentNOsynthesis under physiological oxygen. The FASEB Journal, 2017, 10.1096/fj.201700211R

A. Sabine et al. FOXC2 and fluid shear stress stabilize postnatal lymphatic vasculature. The Journal of Clinical Investigation, 2015, 10.1172/JCI80454

Cell Culture Under Flow: An Overview

Why Cell Culture Under Flow?

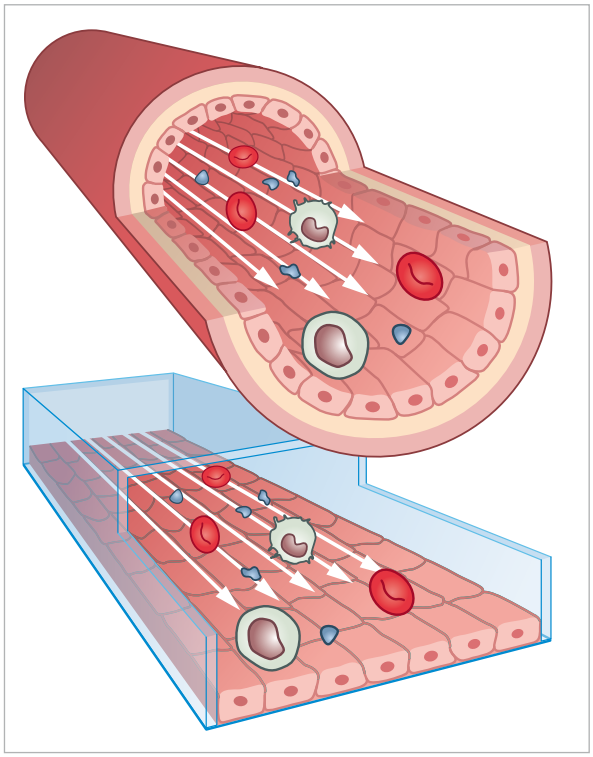
Liquids are a crucial component of every living species. Many cell types are surrounded by moving fluids. Examples are:

- vascular endothelial cells that form the inner layer of blood vessels,
- lymphatic endothelial cells that form the inner layer of lymphatic vessels,
- epithelial cells of the kidney and the lung.

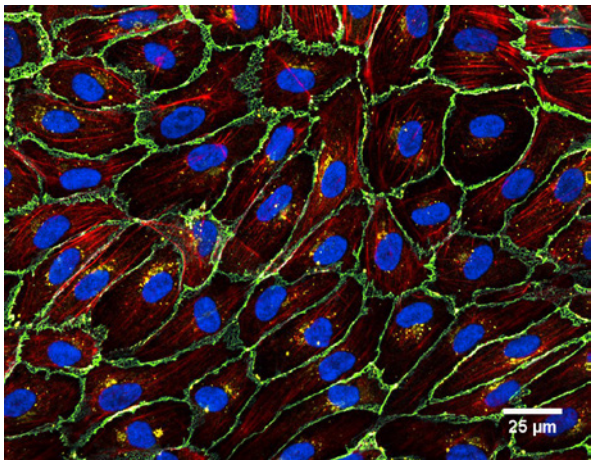
This liquid flow causes shear stress, a mechanical force that influences the cell morphology and behavior in many ways.

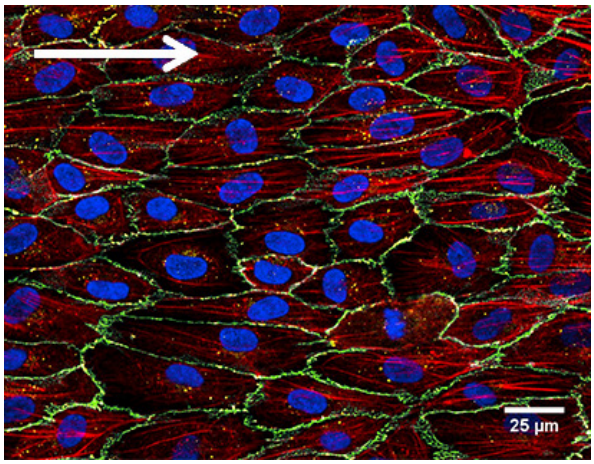
In many standard *in vitro* experiments, cells are cultured without flow. Under these static conditions, shear stress-dependent cellular changes cannot be taken into account. In contrast, in vitro cell culture under flow simulates this mechanical stimulus and induces a more physiological, in vivo-like behavior.

Working under flow conditions can be especially important when using cells that occur in biofluidic systems, such as endothelial or epithelial cells.



Endothelial cells in blood vessels (top) are under continuous flow. Using in vitro cell culture under flow (bottom), these physiological conditions can be simulated.



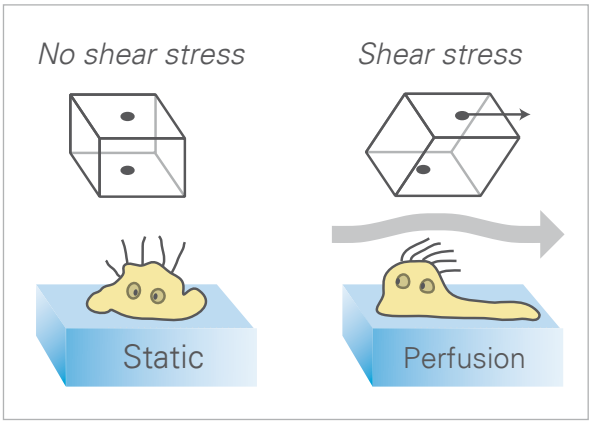


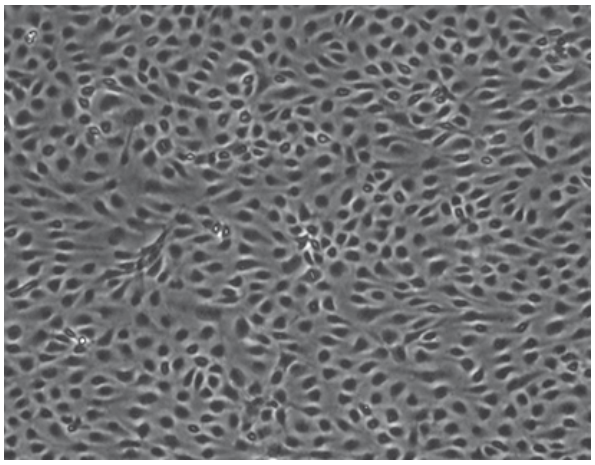
Immunofluorescence of human umbilical vein endothelial cells (HUVEC) under static conditions and under flow. Cytoskeletal F-actin was stained with phalloidin (red). VE-Cadherins (green) mark the adherence junctions. Nuclei are stained using DAPI (blue). Left: HUVEC, static culture, 0 dyn/cm², 5 days, µ-Dish[35 mm, high]. Right: HUVEC, flow-conditioned, 10 dyn/cm², 2 days, µ-Slide I[0.4] Luer.

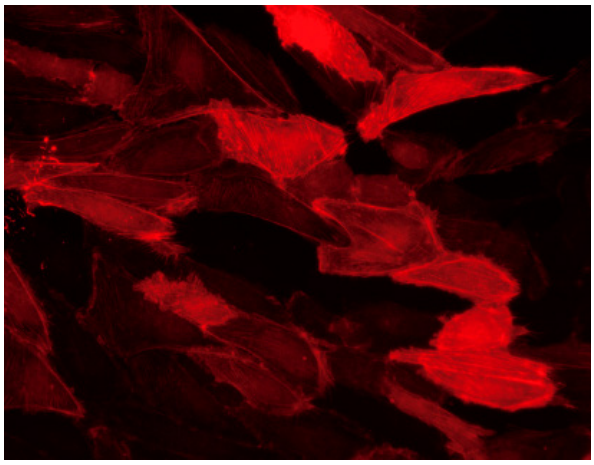
The Impact of Flow/Shear Stress on Cells

Shear stress is the mechanical force induced by the friction of liquid against the apical cell membrane. *In vivo*, several adherent cell types are exposed to mechanical shear stress in biofluidic systems, such as blood and lymphatic vessels or nephrons. This mechanical stimulus has a great impact on the physiological behavior and adhesion properties of cells. Cells react to shear stress by changes in ion channel activation, gene expression, and reorganization of the whole cell layer.

The shear stress is measured in dyne/cm² (dyn/cm²). Physiological shear stress values range from 0.5 to 120 dyn/cm² and depend on the vessel type (e.g., artery or vein), the tissue (e.g., brain, connective tissue, or heart), and the size of the organism (e.g., mouse, rat, or human).







HUVEC cultured under flow conditions (20 dyn/cm²) in a µ-Slide I[0.4] Luer over 9 days. For visualization of the cytoskeleton, the cells were transduced with the adenoviral vector rAV[CMV]-LifeAct-TagRFP 24 hours prior to the experiment.

Vessel	Shear stress (dyn/cm²)	Reference
Aorta	~ 1–22	*C.P. Cheng, R.J. Herfkens, C.A. Taylor. Comparison of abdominal aortic hemodynamics between men and women at rest and during lower limb exercise. J Vasc Surg, 2003, 10.1067/mva.2002.107* *read abstract*
Arteries	~ 10–70	*C.P. Cheng, R.J. Herfkens, C.A. Taylor. Abdominal aortic hemodynamic conditions in healthy subjects aged 50-70 at rest and during lower limb exercise: in vivo quantification using MRI. Atherosclerosis, 2003, 168(2):323–31* *read abstract*
Veins	~ 1–6	*A.M. Malek, S.L. Alper, S. Izumo. Hemodynamic shear stress and its role in atherosclerosis. JAMA, 1999, 282(21):2035–42* *read abstract*
Capillaries	~ 3–95	*A.G. Koutsiaris, et al. Volume flow and wall shear stress quantification in the human conjunctival capillaries and post-capillary venules in vivo. Biorheology, 2007, 44(5–6):375–86* *read abstract*

The Different Types of Flow

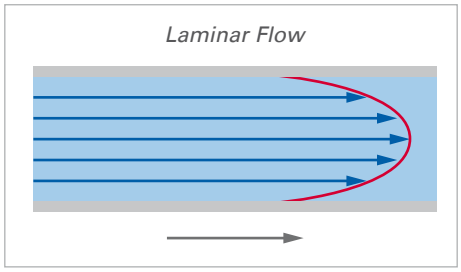
Several types of flow with defined characteristics occur in different tissues and settings. Basically, flow types can be subdivided into laminar flow and turbulent flow:

Type of flow	Physiological occurrence	Flow rate	Flow direction	Generation with ibidi Pump/Slides
Laminar flow	Common, in many healthy vessels			Yes
Unidirectional laminar flow	In most small healthy biological vessels	Constant	Constant	Yes
Pulsatile laminar flow	In large arterial vessels due to fluctuations caused by the heartbeat	Periodically changing	Constant	Yes
Oscillatory laminar flow	Accepted as a means of turbulence simulation using flow chambers	Constant	Periodically changing	Yes
Turbulent flow	Rare, during pathophysiological processes	Changing	Changing	No

Laminar Flow

Laminar flow is defined as the movement of liquids without turbulences. The fluid flows in parallel layers with no disruption between them. Laminar flow can be subdivided into the following:

- Unidirectional laminar flow
- Pulsatile laminar flow
- Oscillatory laminar flow

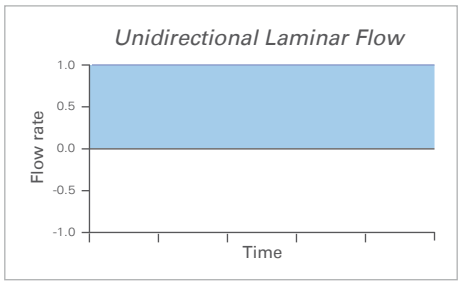


Laminar flow profile. Arrows represent the distribution of velocities.

Unidirectional Laminar Flow

Unidirectional laminar flow is encountered in most small healthy biological vessels, such as small arteries and veins. *In vivo*, certain cells, such as endothelial cells and kidney epithelial cells, are constantly exposed to flow.

Experimentally, unidirectional laminar flow is achieved by perfusing medium through low-walled channels, and by keeping both the flow direction and velocity constant over time.



ibidi Solutions:

- The ibidi Pump System is ideally suited for creating a unidirectional laminar flow.
- The µ-Slide I Luer Family and the µ-Slide VI$^{0.4}$ Channel Slides are recommended for homogeneous unidirectional laminar flow experiments. Using these slides, homogeneous laminar shear stress covers the whole channel area, except for a thin band on either side of the channel walls and near the reservoirs. The width of these bands is proportional to the channel's height.

Non-Uniform Laminar Flow

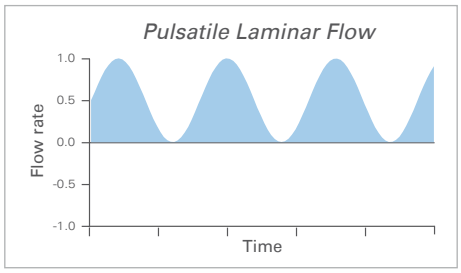
Unidirectional laminar flow can occur in a non-uniform pattern. Here, the flow direction is constant whereas the flow rate spatially varies across the cell layer. *In vivo*, a non-uniform laminar flow occurs at vessel branching sites. Experimentally, non-uniform laminar shear stress can be achieved by a special channel geometry, which generates flow rate variations at specific sites within a slide.

ibidi Solutions:

The µ-Slide y-shaped has been designed for studies of non-uniform flow using the ibidi Pump System. The shear stress level depends on the slide region. For details, please refer to Application Note 18 (PDF).

Pulsatile Laminar Flow

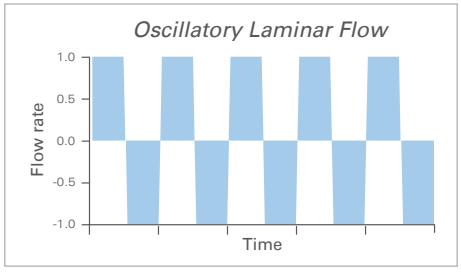
Pulsatile laminar flow is encountered in large arterial vessels due to the fluctuations caused by the heartbeat. Experimentally, this type of flow can be mimicked by employing a flow with a periodically changing flow rate and constant flow direction.



Oscillatory Laminar Flow

Oscillatory laminar flow is used as a means of simulating turbulences. The flow direction is changed at regular intervals. Besides during valve switching, the flow rate is constant.

T. Hosoya, et al. Differential Responses of the Nrf2-Keap1 System to Laminar and Oscillatory Shear Stresses in Endothelial Cells. J Biol Chem, 2005, 10.1074/jbc.M502551200
read abstract

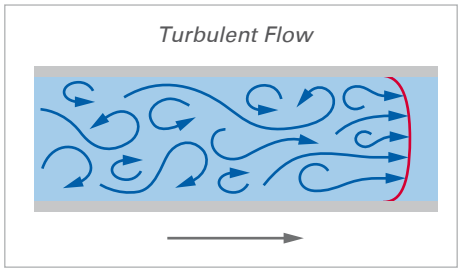


ibidi Solutions:

- The ibidi Pump System is ideal for creating pulsatile or oscillatory laminar flow. Find details in the Instruction Manual for Pulsatile Flow (PDF) and Instruction Manual for the ibidi Pump System (PDF).
- The µ-Slide I Luer family and the µ-Slide VI$^{0.4}$ can be used for pulsatile or oscillatory laminar flow assays.

Turbulent Flow

Turbulent flow is characterized by unpredictable changes in both flow rate and direction over time. *In vivo*, turbulences are rare and can only be found during pathophysiological processes.



Turbulent flow profile. Arrows represent the distribution of velocities.

ibidi Solutions:

Due to physical reasons, turbulent flow cannot be achieved in ibidi flow chambers using physiological flow regimes. Oscillatory laminar flow, which is accepted as a means of simulating turbulences, can be created using the ibidi Pump System. Find the details in the Instruction Manual for the ibidi Pump System (PDF).

Applications of Flow Assays

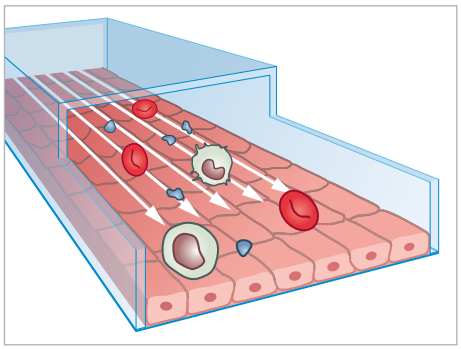
Long-Term Cell Culture Under Flow

Principle

In vivo, endothelial cells develop and differentiate under shear stress conditions. When starting cell-based assays with endothelial cells, you should consider the possible influence of this mechanical force on cell morphology and physiology. To bring the cells to a more physiological, *in vivo*-like state, they are cultured under flow for hours up to several weeks, generating more relevant results. In other words, flow conditioning is crucial for any kind of investigations using cells that are physiologically underlying flow conditions.

Application Examples

- Investigating the influence of shear stress on endothelial cell physiology with various experimental readouts, such as immunofluorescence, western blot, qPCR, and FACS
- Preparing the cell layer for subsequent functional assays, such as rolling and adhesion or transmigration assays



Flow characteristics	Continuous laminar flow, Non-uniform flow, Oscillatory flow (needed for disturbed flow simulation), Pulsatile flow
Experiment duration	Hours, up to several weeks
Recommended pumps	ibidi Pump System
Recommended µ-Slides	µ-Slide I Luer Family µ-Slide VI$^{0.4}$ µ-Slide y-shaped

Rolling and Adhesion Assays

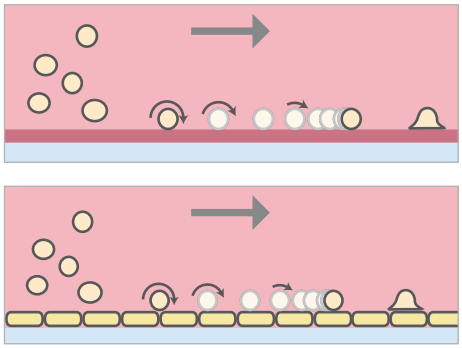
Principle

In a rolling and adhesion assay, leukocytes and/or platelets are perfused over a surface with a protein coating or a cell layer. Their adhesion to and their interaction with the surface can be analyzed under various conditions (e.g., after gene knockdown or drug treatment).

Application Examples

- Investigating the adhesion of platelets and leukocytes on endothelial cells or matrix protein layers

Schulz C, et al. (2009) Novel Methods for Assessment of Platelet and Leukocyte Function Under Flow - Application of Epifluorescence and Two-Photon Microscopy in a Small Volume Flow Chamber Model. Open Biol J 2(1):130–136.
read abstract



Flow characteristics	Continuous laminar flow
Experiment duration	Minutes to hours
Recommended pumps	ibidi Pump System Syringe pump Peristaltic pump
Recommended µ-Slides	µ-Slide I Luer Family µ-Slide VI$^{0.4}$ µ-Slide y-shaped

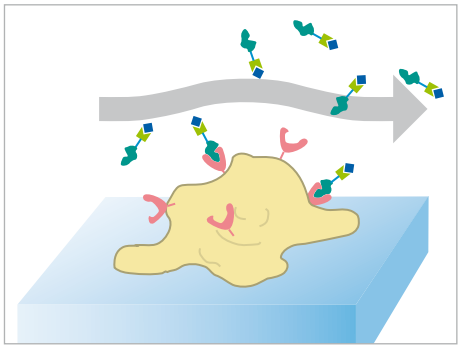
Defined Liquid Exchange

Principle

A channel slide and a pump are used to supply the cells with exactly defined amounts of culture medium and/or any supplement of interest.

Application Examples

- Defined exchange of the cell culture medium
- Live Ca^{2+}-imaging
- Live cell imaging of drug-stimulated adherent cells
- Live cell imaging of cellular stainings



Flow characteristics	Short periods of laminar flow
Experiment duration	Minutes to hours
Recommended pumps	Manual liquid delivery Syringe pump Peristaltic pump
Recommended µ-Slides	µ-Slide VI$^{0.4}$ µ-Slide I Luer Family

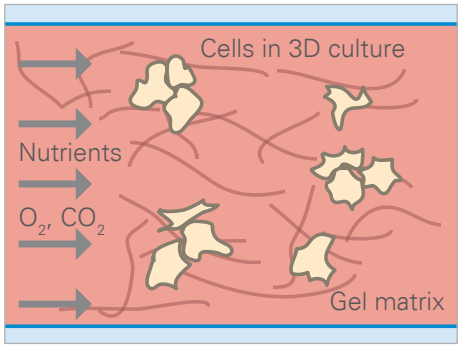
3D Cell Culture

Principle

Cells are cultured in a 3D gel matrix. An interstitial flow is applied directly to the gel to supply the cells with nutrients, O_2, and CO_2 to maintain optimal conditions.

Application Examples

- 3D culture of single cells, spheroids or organoids in a gel matrix (e.g., with hepatocytes, fibroblasts, muscle cells, kidney cells, and stem cells)

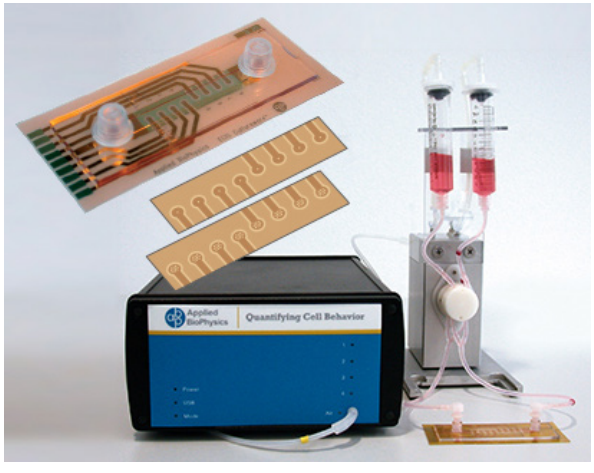


Flow characteristics	Very low interstitial flow
Experiment duration	Hours, up to several weeks
Recommended pumps	ibidi Pump System
Recommended µ-Slides	µ-Slide VI$^{0.4}$ µ-Slide I Luer Family

ECIS Flow Assays

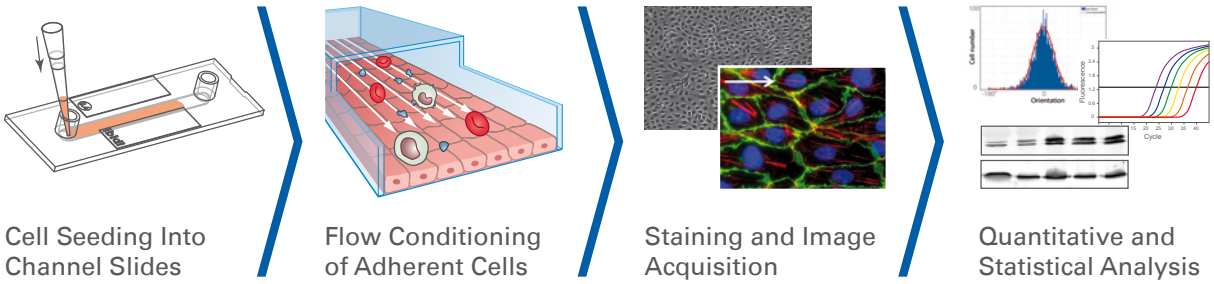
ECIS (Electric Cell-substrate Impedance Sensing) is a platform to measure morphological and physiological changes in an adherent cell layer by means of impedance. Living cells can be measured directly in the vessel without any disturbance, and without requiring any staining. The ECIS Flow Array enables the researcher to combine any type of flow assay with parallel impedance measurement.

Find more details about ECIS flow assays here.



The Flow Assay Experiment: Planning, Setup, and Analysis

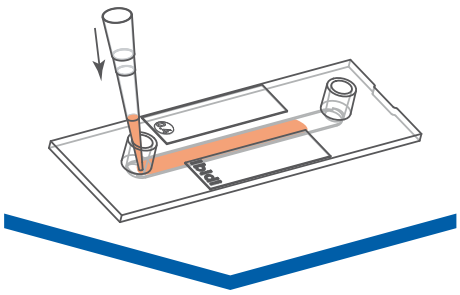
Experimental Workflow



In a flow experiment, *in vivo*-like conditions are mimicked for cells that are physiologically exposed to shear stress (e.g., endothelial cells and epithelial cells).

The ibidi Pump System and the ibidi Channel Slides offer diverse possibilities for conducting flow assays and allow for the adaptation of many experimental parameters to various cellular and environmental requirements.

Sample Preparation



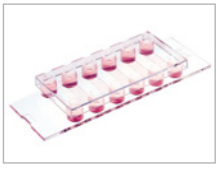
Procedure:

The cell suspension is filled into the channel slide. After cell attachment, the flow experiment can be started. ibidi provides channel slides with different channel heights and geometries, which are suitable for various experimental conditions. For details, please refer to the chapter "The Optimal Perfusion Set and Slide for Your Application".

ibidi Products for This Step


µ-Slide I Luer


µ-Slide VI$^{0.1}$

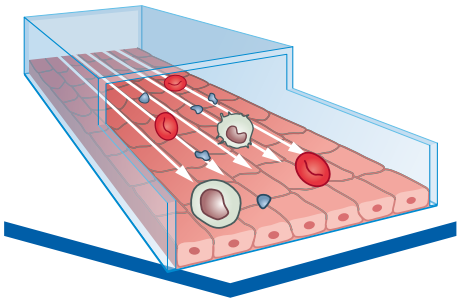

µ-Slide VI$^{0.4}$


µ-Slide y-shaped


sticky-Slide I Luer


sticky-Slide VI$^{0.4}$

Flow Conditioning of Adherent Cells



Procedure

After connecting the channel slide to the ibidi Pump System, the flow can be applied. Under flow, the cell culture medium is continuously pumped through the channel slide. This results in shear stress that influences the cell behavior and physiology. The flow type, flow rate, and the assay duration depend on the experimental setup. Find more details about how to plan the optimal experimental setup in the chapter "Questions to Ask Before Starting an Experiment".

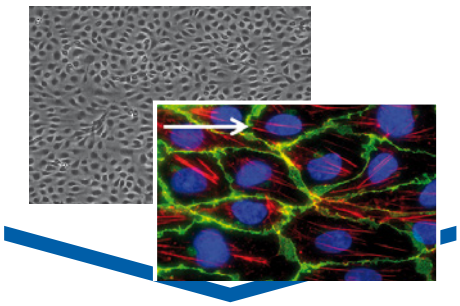
ibidi Products for This Step

ibidi Pump System

ibidi Heating System, Universal Fit

ibidi Gas Incubation System

Staining and Image Acquisition



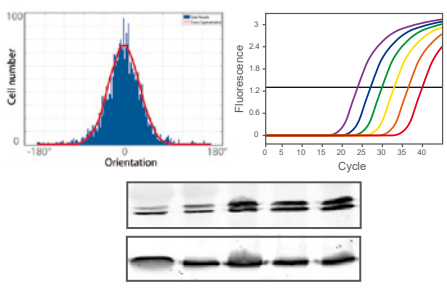
Procedure

Using live cell microscopy, images or videos of the flow-conditioned cell layer can be directly acquired within the channel slide. Further, immunofluorescence stainings and subsequent imaging can be performed directly in the channel slide.

ibidi Products for This Step

ibidi Mounting Medium

Quantitative and Statistical Analysis



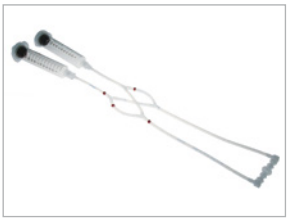
Procedure

After the flow experiment, the cells can be easily analyzed using, e.g., Western blot, qPCR, FACS, and more downstream methods.

Questions to Ask Before Starting an Experiment

These parameters have to be defined in order to set up your experiment correctly:

- Pump type



- Tubing / reservoir size

- Slide type
  including channel geometry and surface/coating

$$\Phi \left[\frac{ml}{min}\right] = \frac{2\,ml \cdot 60\,\frac{s}{min}}{t[s]}$$

- Flow rate
  (see shear stress tables in Application Note 11 (PDF), or use the software calculation)

The following questions help you to define these important parameters:

Which cell type are you planning to investigate?

Knowing your cell type of interest is essential for all subsequent assay planning.

What is the typical shear stress level of your cell type?

The physiological shear stress level of your cell type defines your choice of the pump, tubing, and channel slides.

Which kind of shear stress do you want to investigate?

Knowing the type of shear stress that will be applied to your cells is important for choosing the optimal perfusion system for your assay. Please have a look at the different types of shear stress and suitable pump systems here.

Which cell culture medium are you planning to use? Which viscosity does this medium have?

The viscosity of the medium is important for shear stress calculation. Please find an exact description of shear stress calculation in the Application Note 11: "Shear Stress and Shear Rates for ibidi µ-Slides" (PDF).

What is the available amount of medium, medium supplements, and number of cells?

The amount of medium/supplements and the number of cells that are available for the flow assay, influence your choice of the reservoir and tubing size. For more details, please have a look at the Perfusion Set Selection Suide.

How long will the experiment last (hours, days, weeks)?

The duration of an experiment is important for choosing the perfusion system that fits your needs best. Please have a look at the different pump systems and their applications here.

Should your assay be a one-way setup with defined medium conditions, or a circular setup, where the medium is slowly conditioned by the cells?

The type of setup determines the choice of a suitable pump. Please have a look at the different pump systems and their applications here.

Which coating is required for your cells to adhere optimally?

The coating of the channel slide should enable optimal adherence and growth of your cell type.

Are the reagents to be used expensive or inexpensive? Are they available in large volumes?

The availability of reagents and their costs are an important factor for choosing the size of the tubing and reservoir. For more details, please have a look at the Perfusion Set Selection Guide.

What are the endpoints of the experiment (e.g., qPCR, western blot, FACS, immunofluorescence staining)?

The method of endpoint analysis is important for calculating the needed cell number, which influences the choice of the used cell culture vessel.

Choosing the Optimal Setup for Your Experiment

The Optimal Pump System for Your Experiment

Several pump systems are available on the market. Their different properties make them suitable for specific experimental requirements.

A **syringe pump** consists of a syringe, which is mounted onto a device that moves the plunger in a defined velocity. The outlet of the syringe can be connected to a tubing and a slide to create a flow in the slide. This easy-to-use device is mainly suitable for short-term experiments, where a relatively low medium volume and a slow to moderate flow rate are needed. More complex devices can also generate a circulating medium flow over the cells.

A **peristaltic pump** consists of a rotor with several contact points to the inserted tubing. The coils squeeze the tubing and thus move the medium forward. The peristaltic pump is suitable for long-term flow in parallel flow chambers.

The ibidi Pump System generates a flow by applying an air pressure onto the medium-filled reservoirs. By recirculation of the medium using a special switching pattern , a constant unidirectional flow is generated. This makes it an ideal setup for applying defined shear stress in long-term cell culture. Using the ibidi Pump System, it is possible to simulate continuous and pulsatile laminar flow, as well as oscillatory flow.

To help you find the pump system that fits your needs, please see the properties of the different pump systems, and whether they are applicable for your flow type of choice, in the tables below:

Possible Flow Types

	Syringe pump	Peristaltic pump	ibidi Pump System
Circulating flow	Yes (only with push-and-pull pumps)	Yes	Yes
Long-term unidirectional flow	Yes (limited by volume)	Yes	Yes
Short-term flow	Yes	Yes (with limitations)	Yes (with limitations)
Pulsatile flow for simulating heartbeats	No	No	Yes
Oscillatory flow for simulating turbulences	Yes	No	Yes

Pump System Characteristics

	Syringe pump	Peristaltic pump	ibidi Pump System
Pulsation	Almost none (only initial pulse)	Yes (undefined pulsation via drive shaft, depending on rotor type)	Almost none (only during valve activation)
Mechanical stress during pumping on non-attached cells held in a reservoir	Almost none (sedimentation can be a problem)	Strong	Very low
Combinable with microscopy	Yes	Difficult due to pulsation	Yes
Parallel experiments possible	Yes	Yes	Yes
Setup within an incubator	Difficult	Difficult	Yes
Long-term experiments possible with low medium volume	No	Yes	Yes
Programmability	Yes	Yes	Yes

If you have further questions, please contact ibidi or your local distributor for a personal consultation.

The Optimal Shear Stress for Your Experiment

Depending on the vessel type and tissue, the shear stress in a human body varies between almost 0 and over 100 dyn/cm². Information about the physiological shear stress that applies for your cell type of interest can be found in the chapter "The Impact of Flow / Shear Stress on Cells" and in the literature.

It is crucial to perform preliminary experiments to determine at which shear stress level and after which time of conditioning the investigated protein or pathway reacts on the shear stress stimulus. Since the restructuring of the cell layer takes at least several days, it might be necessary to prolong the conditioning to such a long time frame.

Find detailed information about the shear stress-regulated factors and the corresponding response times in this review:
P.F. Davies. Flow-Mediated Endothelial Mechanotransduction. Physiological Reviews, 1995, 10.1152/physrev.1995.75.3.519
read abstract

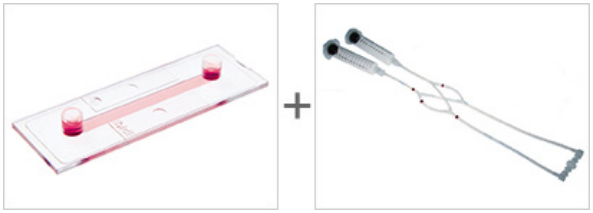
The Optimal Perfusion Set and Slide for Your Application

ibidi provides several Channel Slides with different volumes and geometries. These can be combined with the different ibidi Perfusion Sets, which are offered with varying inner diameters and tube lengths.

With each combination of a Perfusion Set and Channel Slide, a specific shear stress range can be achieved using the ibidi Pump System. Depending on the shear stress your setup requires, choose a suitable combination according to the Perfusion Set and µ-Slide Selection Guide.

General rules:

- Higher shear stresses are created in Channel Slides with lower channels.
- Lower shear stresses are created with smaller inner diameters in the tubing.



Explore the detailed Perfusion Set and µ-Slide Selection Guide.

Perfusion Set and µ-Slide Selection

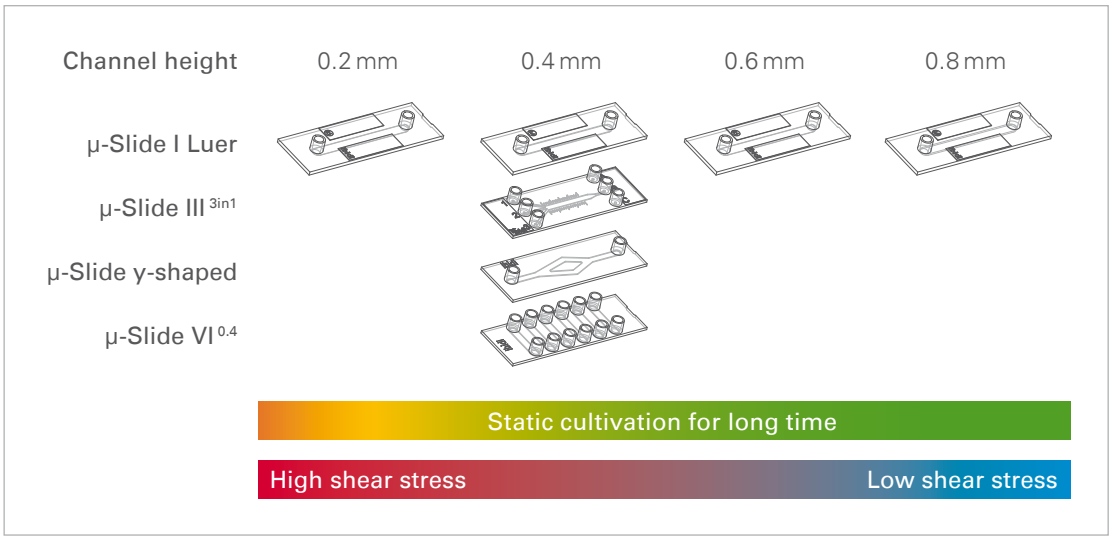
	Red (ID 1.6 mm)	Yellow/Green (ID 1.6 mm)	Blue (ID 0.8 mm)	White (ID 0.8 mm)	Yellow (ID 0.5 mm)	Black (ID 0.5 mm)
Low shear stress			✓	✓		
High shear stress	✓	✓			✓	✓
Long tubes for microscopy (50 cm)		✓		✓		✓
Working volume (ml)	12	12	12	12	2.5	2.5
Standard reservoir* (ml)	10	10	10	10	2	2

** By changing the reservoir, different working volumes can be achieved. Reservoirs are available with a syringe volume of 2, 12, and 50 ml.*

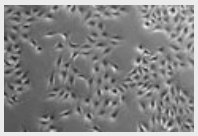
Suitability of Channel Slides for Different Shear Stress Ranges

The suitability of Channel Slides for different shear stress ranges depends on the channel height.

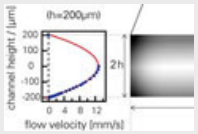
Please note: due to the very low channel height of 0.2–0.8 mm, the suitability of the Channel Slides for static cell culture is limited. Demanding, fast proliferating cells might be starving already after several hours if no fresh medium is added!



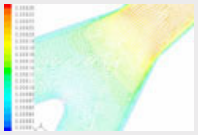
Application Notes



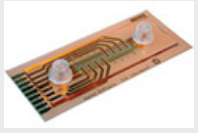
AN 03: Growing Cells in µ-Channels (PDF)
Growing cells inside a µ-Slide VI$^{0.4}$, and a comparison between channels and open wells



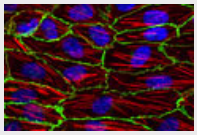
AN 11: Shear Stress and Shear Rates (PDF)
Detailed information on shear stress/shear rates and flow rates in our channel slides



AN 18: Shear Stress and Shear Rates in µ-Slide µ-Shaped (PDF)
Detailed information on shear stress/shear rates and flow rates in our µ-Slide y-shaped



AN 48: Shear Stress and Shear Rates in ECIS Flow Arrays (PDF)
Detailed information on shear stress, shear rate, and flow rate calculations in ECIS Flow Arrays



AN 13: HUVECs Under Perfusion (PDF)
Setting up a flow experiment using µ-Slide I and HUVECs

AN 25: Serial Connection of Flow Chambers (PDF)
Setup protocol for connecting several Luer-Slides to one Fluidic Unit

AN 31: Serial Connection of µ-Slide VI$^{0.4}$ (PDF)
Protocol for connecting the six channels of µ-Slide VI$^{0.4}$ to one Fluidic Unit

Cells Under Flow: Experimental Examples

Immunofluorescence Staining of Flow-Conditioned Endothelial Cells

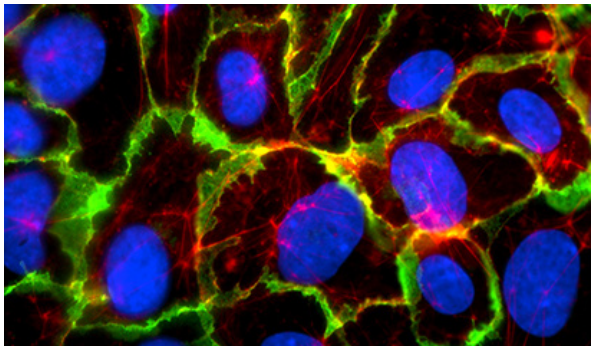
After the flow experiment, immunofluorescence stainings can easily be done in the ibidi Channel Slides. When comparing the cultivation of HUVEC under static and flow conditions, the differences in the cellular organization and various cellular compartments (e.g., tight junctions) are clearly visible.

Find detailed information about immunofluorescence staining in ibidi µ-Slides and µ-Dishes here.

Adherence Junctions (VE-Cadherins)

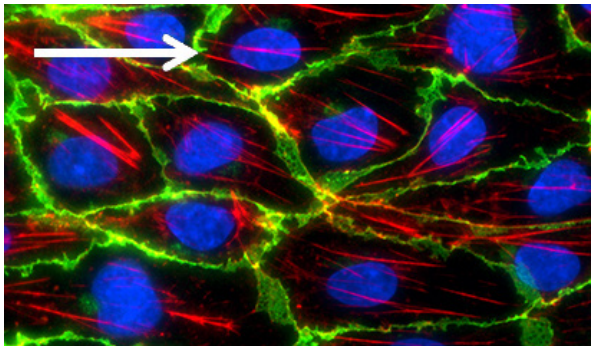
Under static conditions, HUVEC are generally large with a chaotically structured actin skeleton. In contrast, flow-conditioned cells are elongated and show distinct stress fibers. F-actin was stained with phalloidin (red). VE-cadherins (green), which mark the adherence junctions, are present in both conditions. Nuclei are stained using DAPI (blue).

Static culture



HUVEC, static culture, 0 dyn/cm², 5 days, µ-Dish 35 mm, ibiTreat.

Flow culture

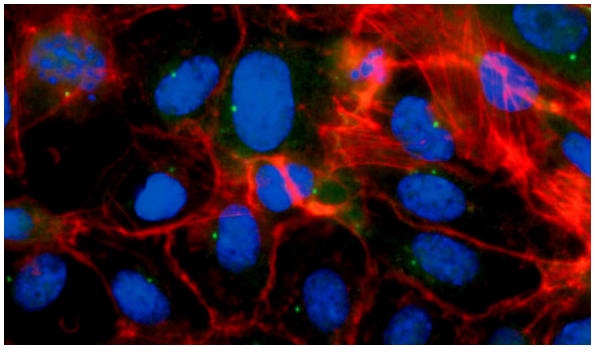


HUVEC, flow-conditioned, 10 dyn/cm², 5 days, µ-Slide I 0.4 Luer, ibiTreat.

Tight Junctions (Claudin-5)

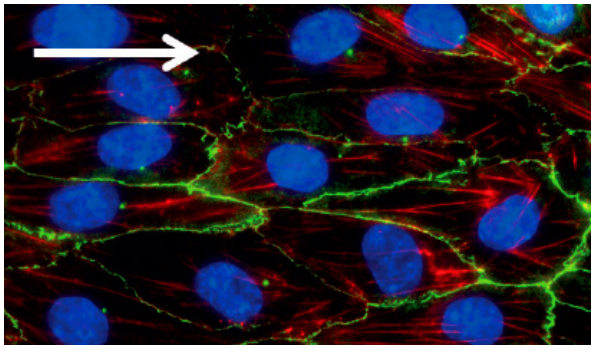
Claudin-5, a tight junction protein, can be found at the cell-cell contact zone of flow-conditioned cells after 5 days (green). This shows that the impact of the mechanical shear stress is crucial for the differentiation of the cell layer. F-actin was stained with phalloidin (red). Nuclei are stained using DAPI (blue).

Static culture



HUVEC, static culture, 0 dyn/cm², 5 days, µ-Dish 35 mm, ibiTreat.

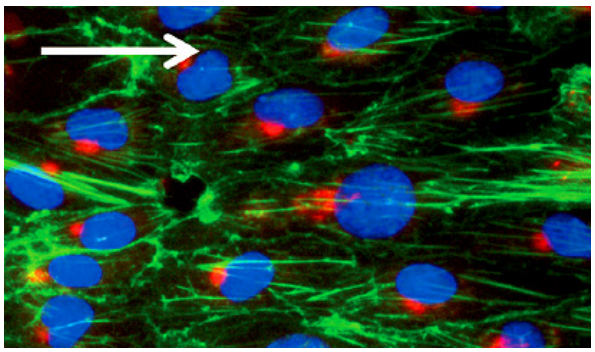
Flow culture



HUVEC, flow-conditioned 10 dyn/cm², 5 days, µ-Slide I 0.4 Luer, ibiTreat.

Golgi Apparatus

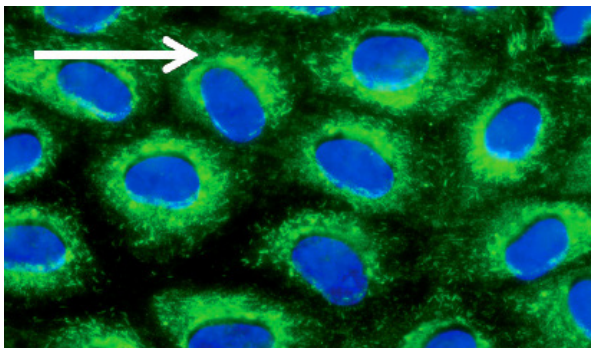
The Golgi apparatus, stained with Anti-Human-Golgin-97 (red), is localized along the direction of flow. F-actin was stained with phalloidin (green). Nuclei are stained using DAPI (blue).



HUVEC, flow-conditioned, 10 dyn/cm², 4 days, µ-Slide I$^{0.4}$ Luer, ibiTreat.

Von-Willebrand-Factor

The von-Willebrand-Factor (vWF, stained with anti human von-Willebrand-Factor) is a typical endothelial cell membrane marker. When the cells are exposed to flow, the vWF-multimers elongate to rods that are sticking to the cell membrane (green). Nuclei are stained using DAPI (blue).



HUVEC, flow-conditioned, 10 dyn/cm², 5 days, µ-Slide VI$^{0.4}$, ibiTreat.

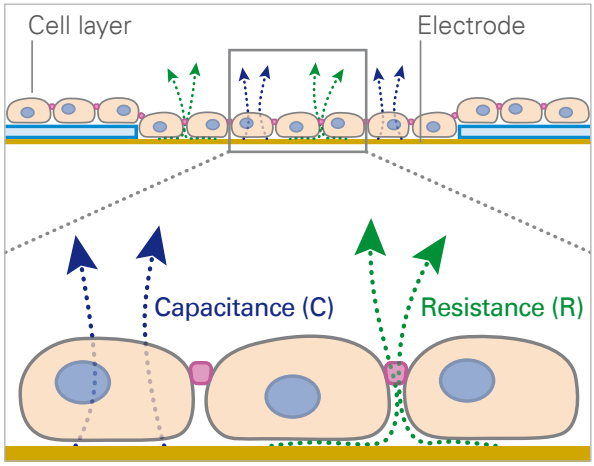
Impedance Measurements Under Flow Stimulation

Mechanical perturbations have a profound effect on the characteristics of cell layers in vitro. In long-term experiments with HUVEC, three different phenotypes can be observed: a round flat cell after seeding, elongated cells after 1–2 days, and finally an oriented cobblestone appearance of a dense, compact cell layer. The morphological changes are accompanied by physiological changes of the endothelial cell monolayer, which can be measured by impedance monitoring.

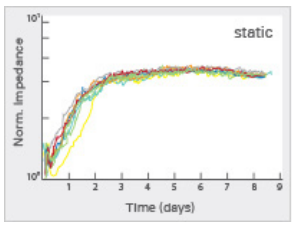
Working Principle of Impedance Measurements

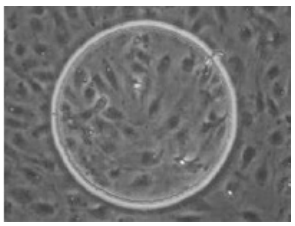
Cells are cultivated in channels with electrode arrays. Depending on the state of development of the endothelial cell monolayer, the gaps between the cells (influenced by the cell-cell contacts) change in size. These shifting gaps can be characterized by their change in conductivity when applying AC currents of different frequencies. The continuous red lines in the scheme represent the ion currents between the cells when applying the AC current.

For a detailed description, please refer to the applications website "Impedance-Based Cell Assays".



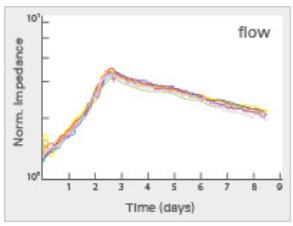
Static culture

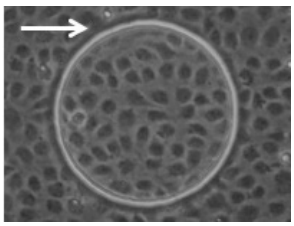




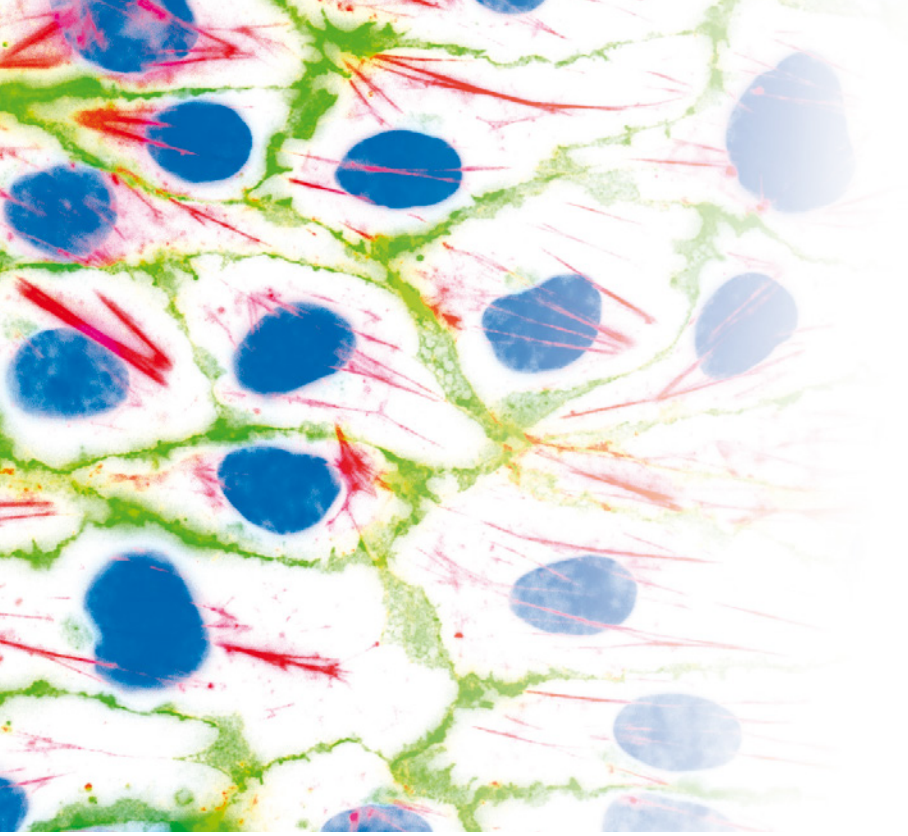
After seeding, cells grow to confluence in 2–3 days. During this time, the conductivity is reduced, and the resistance rises to a plateau, which is then maintained under the subsequent static culturing over several days.

Flow culture





Applying shear stress reduces the conductivity and increases the impedance of the monolayer. Over a period of days the impedance of the endothelial monolayer decreases. The physiological properties of the cell monolayer are altered when compared to the static conditions.



ibidi®
cells in focus

Manufacturer

ibidi GmbH
Lochhamer Schlag 11
82166 Gräfelfing
Germany

Toll free within Germany:
Phone: 0800/00 11 11 28
Fax: 0800/00 11 11 29

International calls:
Phone: +49 89/520 46 17-0
Fax: +49 89/520 46 17-59

E-Mail: info@ibidi.com
ibidi.com

North American Headquarters

ibidi USA, Inc.
2920 Marketplace Drive
Fitchburg, WI 53719
USA

Toll free within the US:
Phone: +1 844 276 6363

International calls:
Phone: +1 608 441 8181
Fax: +1 608 441 8383

E-Mail: ibidiusa@ibidi.com
ibidi.com

FL_AG_034, V 1.1 2019/06

For free samples, application notes, and handling movies, please visit us at:

ibidi.com