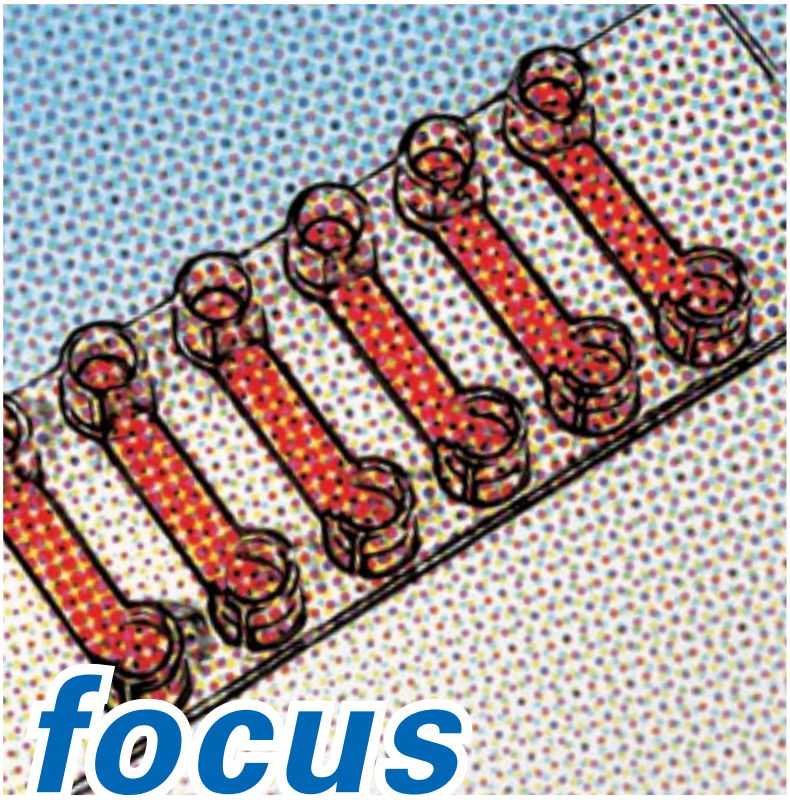
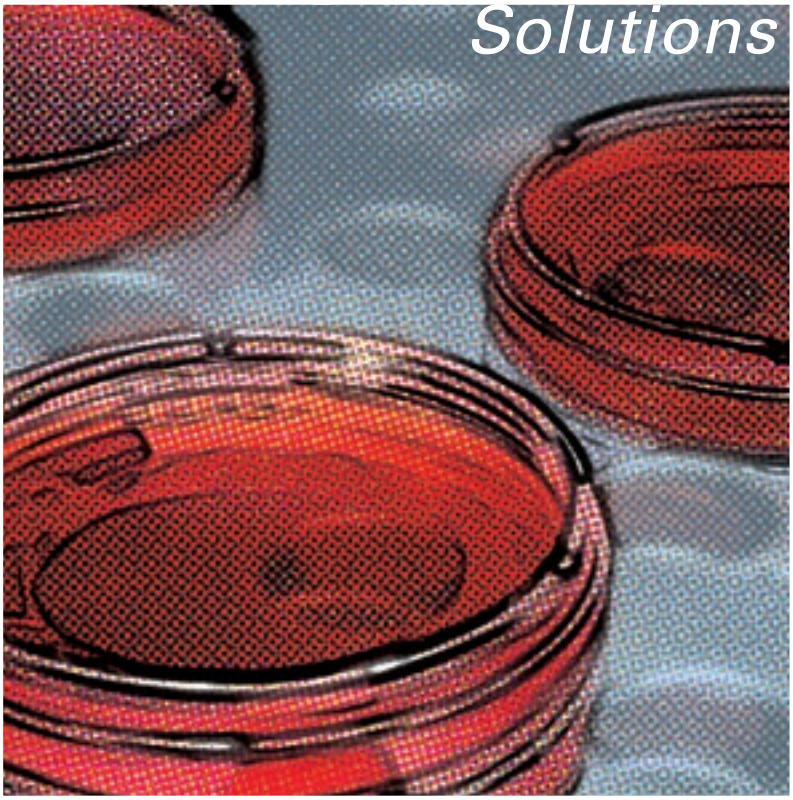
ibidi®

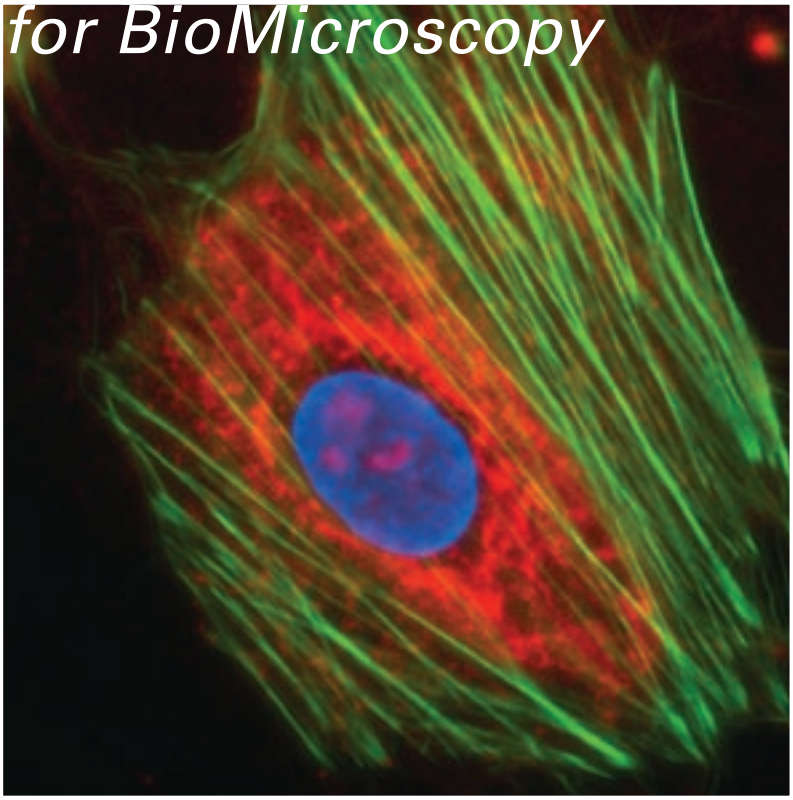
cells in focus



cells in focus

Solutions for BioMicroscopy

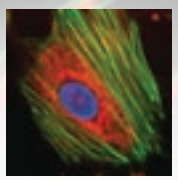




Cell Culture & Microscopy · Immunofluorescence
Live Cell Imaging · Video Microscopy · Cell Based Perfusion Assays
Chemotaxis · Angiogenesis · Wound Healing and Invasion

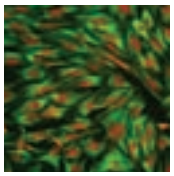
2010

cells in focus


Cell microscopy

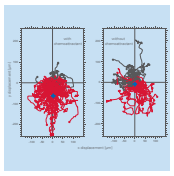
- perfect cell growth
- high imaging quality


Immunofluorescence

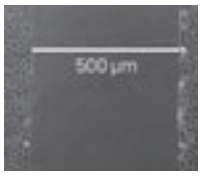
- small volume of 25 µl
- parallel screening assays

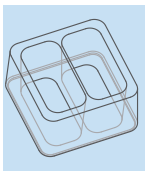




Chemotaxis assays

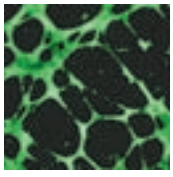
- stable linear gradients
- 48 hour cell tracking possible

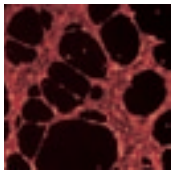





Wound healing & Migration

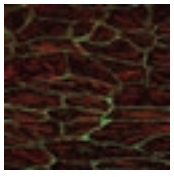
- defined separated areas
- highly reproducible





Angiogenesis assays

- sprouting & tube formation assays
- 3D gel matrix


Flow assays

- defined flow rates
- rolling and adhesion

Instruments & Accessories

ECIS Technology

page 104

Electric Cell-substrate Impedance Sensing (ECIS) is an automated system to monitor the cellular behavior.

The Extracellular Flux System

page 106

Determines cellular bioenergetics in a microwell plate and measures the two major energy yielding pathways – aerobic respiration and glycolysis.

EVOS Microscopes

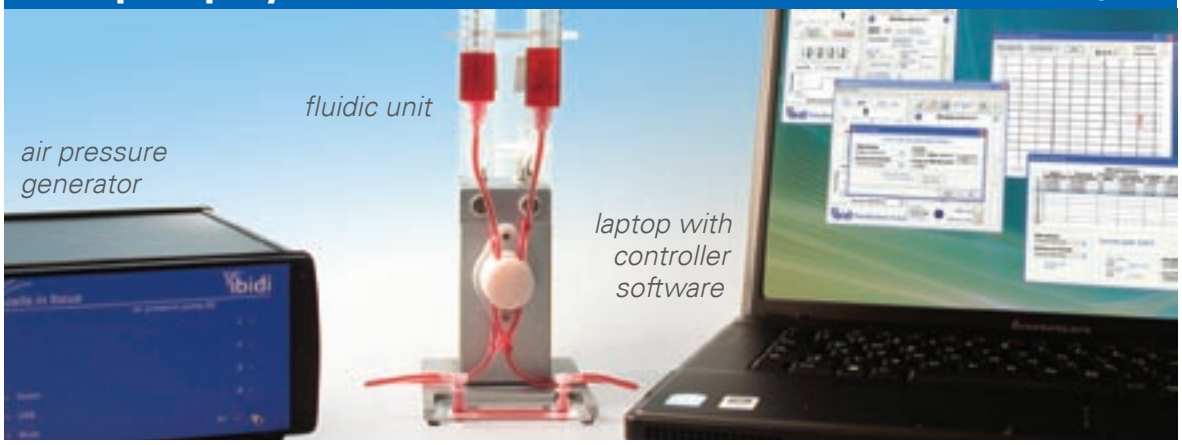
page 108

The new microscope series from AMG. As xl version for phase contrast or fl version for fluorescence based applications.

Instruments by ibidi

ibidi pump system

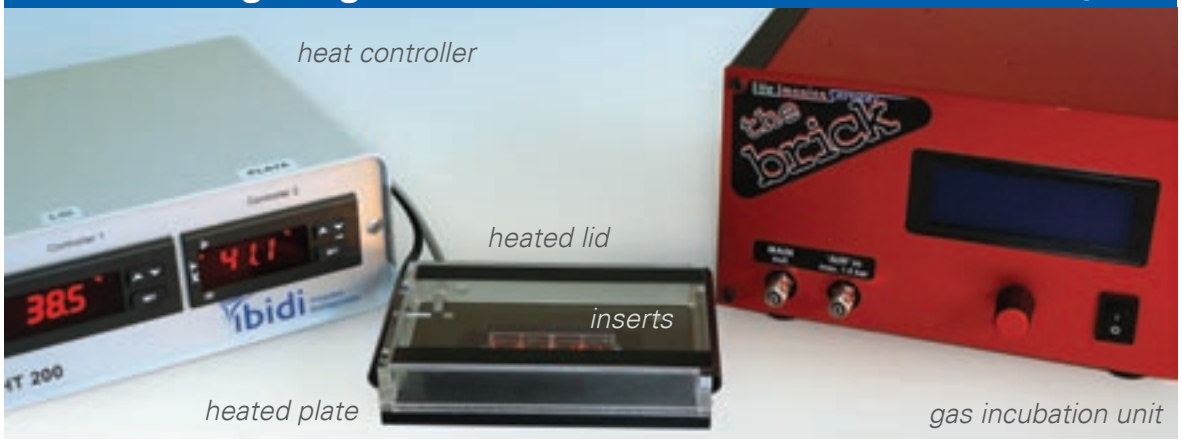
page 96



The ibidi pump system is designed to cultivate cells under flow conditions, inside an incubator in long term assays.

ibidi heating stages and incubation units

page 100



The universal, modular system fits all inverted microscope platforms and upgrades a microscope to a live cell imaging unit.

Kits, reagents & accessories

page 87

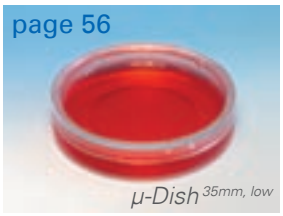
ibidi now offers a fine selection of kits and accessories all linked to microscopy and cell based assays.

Disposables

page 56

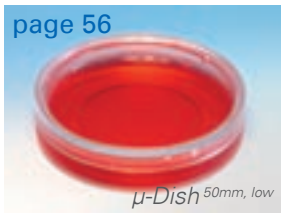
35 mm Petri dish with a thin bottom for high end microscopy – with high walls – also available w. glass bottom

page 56



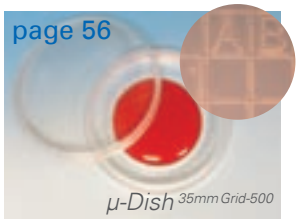
35 mm Petri dish with a bottom for high end microscopy – with low walls for micromanipulation

page 56



50 mm Petri dish with large cultivation area and for micro-manipulation

page 56

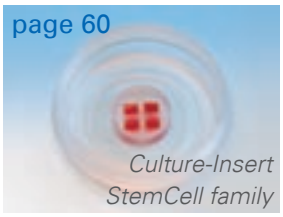


35 mm Petri dish for relocating cells on a 500 µm grid

page 58

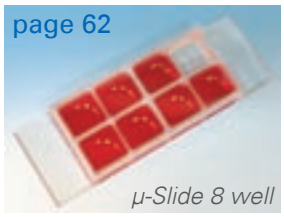
Silicon-insert for wound healing and 2D invasion assays

page 60



4-well silicon-insert for stem cell cultivation and long term micros-copy studies

page 62

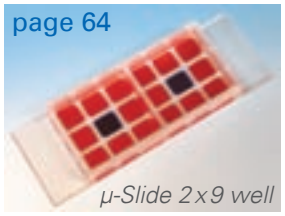


µ-Slide with 8 wells for immuno-fluorescence and long term cultivation

page 63

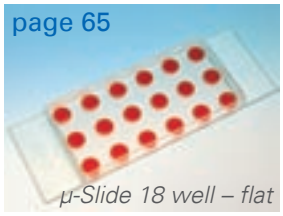
µ-Slide for cell microscopy on or in 3D-gel matrices, providing flat gel surfaces with a minimum of gel used

page 64



µ-Slide with 2 major chambers each with 9 subdivisons for co-cultivation assays

page 65

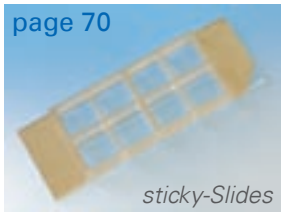


µ-Slide with 18 wells for matrix tests or spotting samples like in RNA assays

page 67

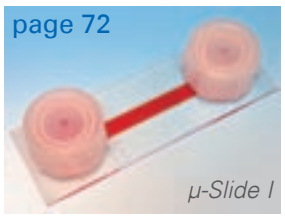
Removable silicone chamber for cell culture and immuno-fluorescence stainings

page 70



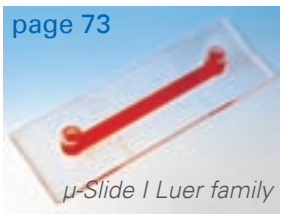
Slides without any bottom but with self-adhesive underneath for attaching your own substrate

page 72



Combination of a cell culture chamber and a coverslip for imaging inside a channel

page 73



µ-Slide I Luer family for easy flow applications in a channel – also available as Flow Kit –

page 74

6 channel µ-Slide for multiple im-munofluorescence stainings – also available as Flow Kit –

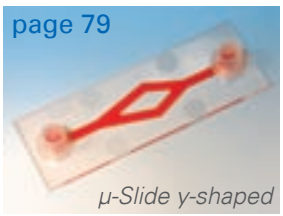
page 76

µ-Slide for stable gradients, 3 chambers on one slide for video microscopy chemotaxis assays

page 78

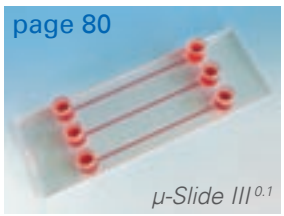
µ-Slide that addresses upright microscopy, applicable in static or perfusion assays

page 79



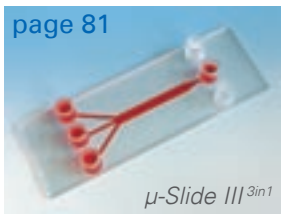
Flow through µ-Slide for bifurcation studies & simulation of blood vessels – also available as Flow Kit –

page 80



3 assyas on one µ-Slide; for perfu-sion with rare cells or mouse blood – also available as Flow Kit –.

page 81

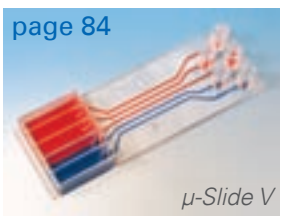


µ-Slide that merges 3 separated liquids into one channel in a laminar flow – also available as Flow Kit –

page 84

µ-Slide with 6 parallel channels for short term assays

page 84



Protein assay µ-Slide for parallel solid phase immunoassays with extremely low sample amounts

page 69

96 quadratic wells black plate with flat and clear bottom

page 69

Quadratic wells for high through-put applications in cell based assays

Table of contents

Contents

In addition to this catalog comprehensive up-to-date information can be found on our website www.ibidi.com, including:

Products

- find the latest products
- updated information

FAQs

- prevention of evaporation
- use correct immersion oils
- solvent compatibility

References

- more than 300 publications
- screen database
- get a free box if you publish
- for images used in this catalog

Product pages

- instructions
- detailed product information
- application notes
- movies

Application notes

- find experimental details for complex applications like chemotaxis and perfusion assays
- immunofluorescence protocols
- coatings of µ-Slides and much more

Distributors

- ibidi is represented in more than 25 countries
- find contact details for your country

7 Good reasons to use ibidi µ-Slides

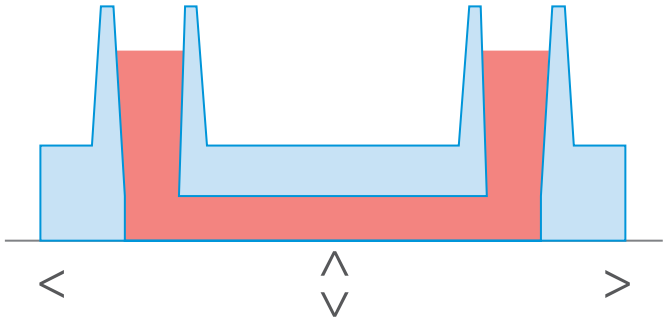
1

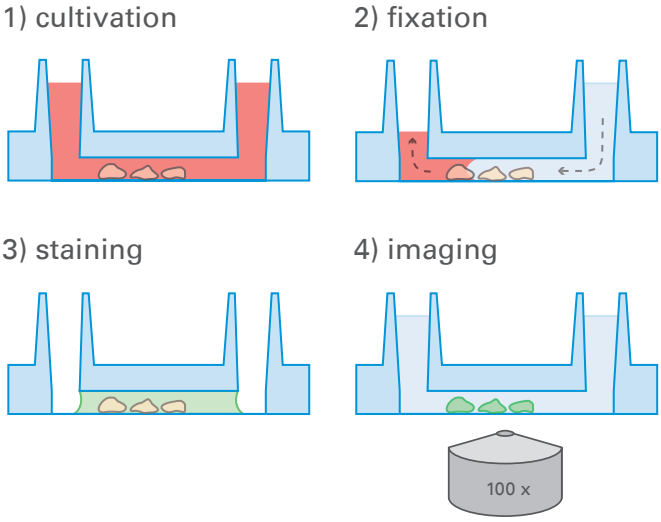
Optical grade base

with thickness of a standard glass coverslip (180 µm, No. 1.5)

Standard dimensions

dimensions of all ibidi µ-Slides correspond to standard microscopy glass slides (75.5 x 25.5 mm)





2

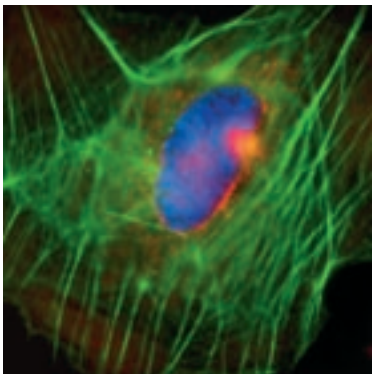
all-in-one chamber

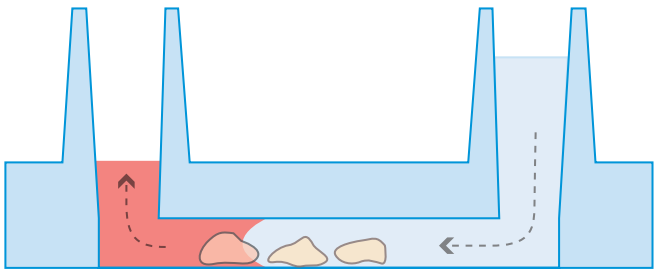
- *in situ* cell experiments without cell transfer
- perfect immunofluorescence staining chamber

3

ibidi plastic material

- perfectly suited for immunofluorescence staining
- biocompatible plastic without glue
- no autofluorescence





4

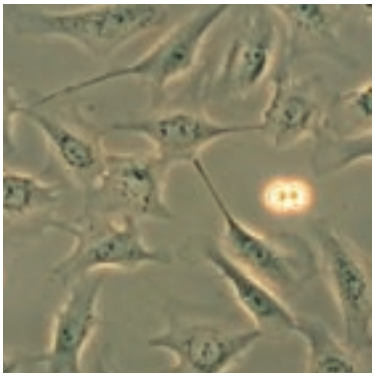
Resistant to all fixation methods

material resistant to methanol, acetone, para-formaldehyde, and acids

5

Tissue culture treated (ibiTreat)

- superb cell growth on the tissue culture treated surface
- individual coatings possible



6

World class tech support

Discuss your questions:

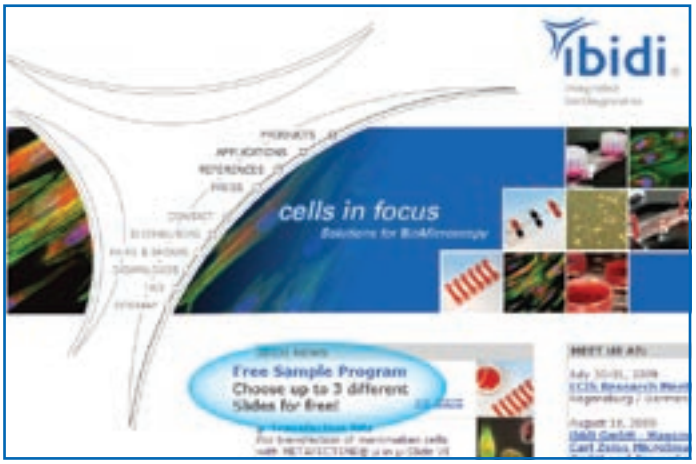
- Which immersion oil to use?
- How to prevent evaporation?
- How to perform a chemotaxis assay?

7

Free sample program

Experience it for yourself with a free trial of our µ-Slides and µ-Dishes via our website:

www.ibidi.com

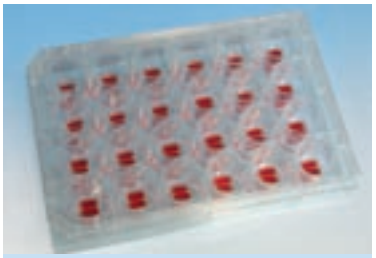


New products in 2010

Culture-Insert StemCell

4-well silicon-insert for stem cell cultivation and long term microscopy studies

page 60



Culture-Insert 24

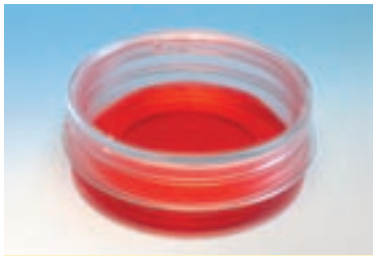
Our Culture-Inserts – now ready to use in 24 well plates for parallel screenings

page 59

sticky-Slides

Slides without any bottom but with self-adhesive underside to assemble your own systems

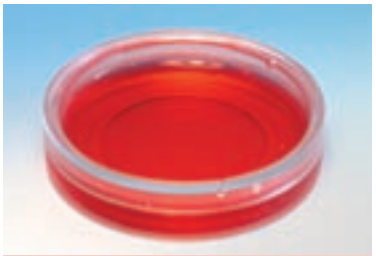
page 70



µ-Dish 35mm, high glass bottom

35 mm Petri dish with a glass bottom for TIRF and single photon measurements

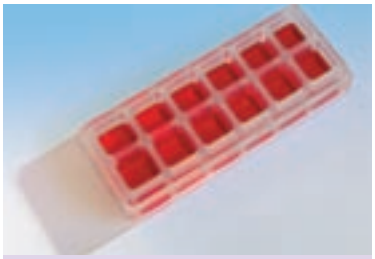
page 57



µ-Dish 50mm, low

50 mm Petri dish with large cultivation area and for micromanipulation

page 57



µ-Chamber 12 well

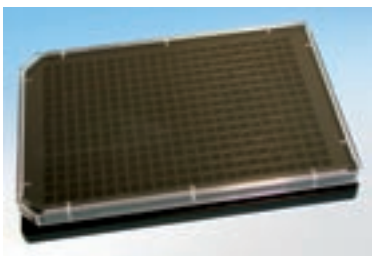
Removable silicone cultivation chamber for cell culture and immunofluorescence stainings

page 67

DIC lid

For perfect images in 35 mm µ-Dishes using DIC

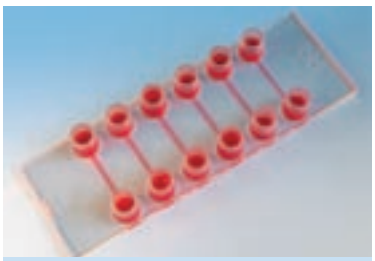
page 56



µ-Plate 384 well

Quadratic wells for high throughput applications in cell based assays

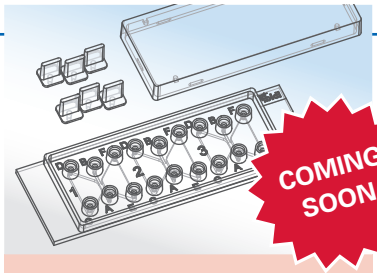
page 69



µ-Slide VI 0.1

6 channel µ-Slide for flow assays with minimal volume

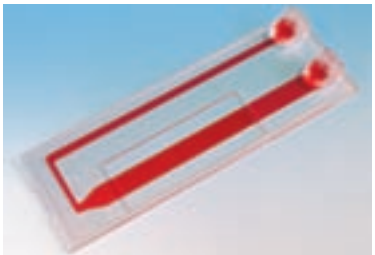
page 74



µ-Slide Chemotaxis 3D

Investigate chemotaxis of non-adherent cells in gel matrices

page 77



µ-Slide upright 0.7

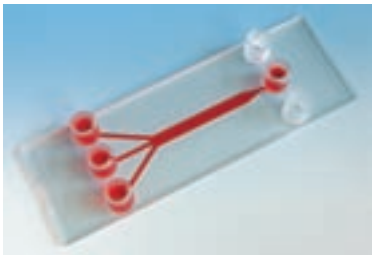
µ-Slide that addresses upright microscopy, applicable in static or perfusion assays

page 78

µ-Slide III 0.1

3 assays on one µ-Slide; for perfusion with rare samples such as cells or blood

page 80



µ-Slide III 3in1

µ-Slide that merges 3 separated liquids into one channel in a laminar fashion

page 81

Elbow Luer connector

Connectors in an elbow style for all µ-Slides Luer

page 82

µ-Slide I Luer electrode

µ-Slide I Luer electrode for microscopic and electric analysis of cells simultaneously

page 86

ibidi Mounting Medium

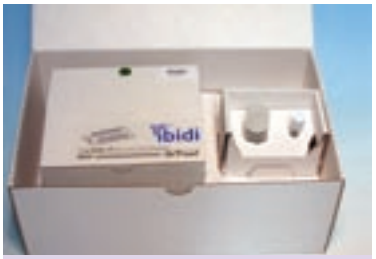
Mounting medium optimized for fluorescence microscopy

page 87

Immersion oil

An oil fully compatible with high end imaging and ibidi plastic material

page 89



µ-Transfection Kit VI

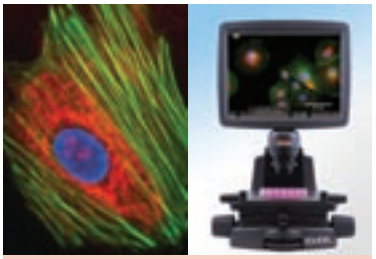
For transfection of mammalian cells in µ-Slide VI

page 88

Extracellular Flux System

Seahorse system to determine bioenergetics in a microplate

page 106

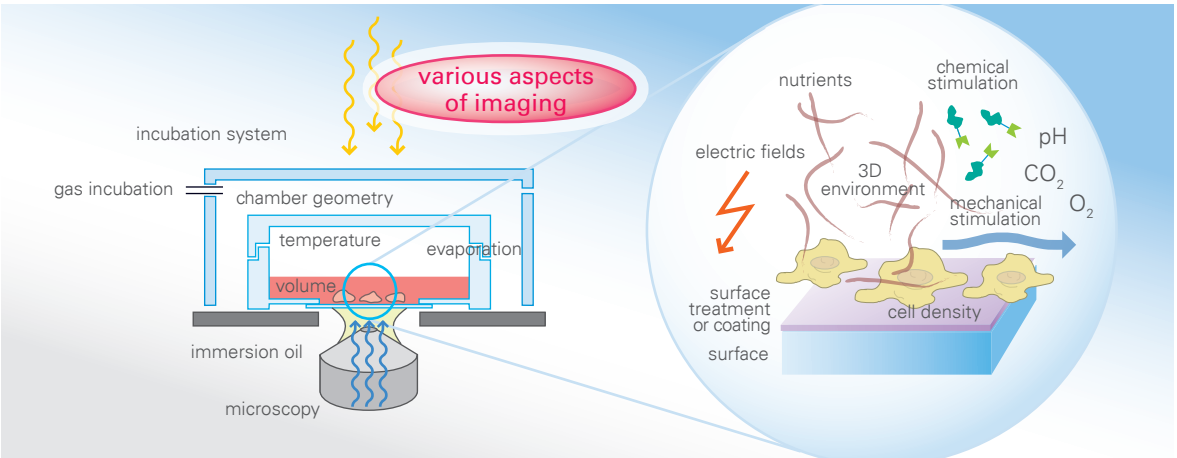


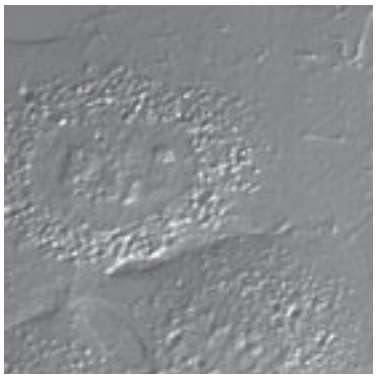
EVOS Microscope

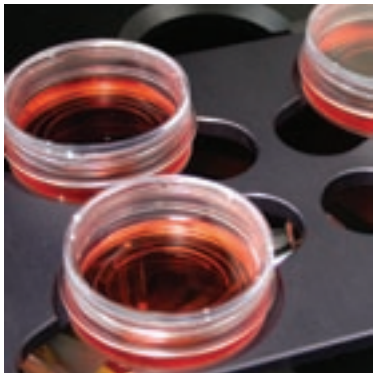
Perfect images in phase contrast and/or fluorescence mode, also available as complete live cell imaging setup

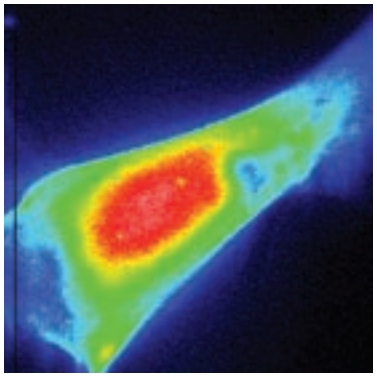
page 108

Cell culture & microscopy









Imaging today

During the last decade, imaging of cultured or primary cells played a major role in understanding biological processes. Modern imaging technologies allow for the visualization of multi parameter data sets. Furthermore, beside the spatial resolution time- or wavelength-shifts can also be measured, providing a deeper insight into processes on a subcellular level.

Now, the development of these novel microscopy techniques allows scientists to analyze cell based assays directly. This had a remarkable impact on cell and developmental biology, as well as for neuroscience, biophysics, and diagnostics. The µ-Slide (micro Slide) family is the answer to the demands in these high end research fields, as it combines a chamber devoted to cell based assays with a high resolution microscopy support.

From immunofluorescence ...

Cell growth in ibidi µ-Slides has been thoroughly studied. More than 300 research articles have been published, where µ-Slides have been used successfully. Even the smallest channel allows perfect cell growth and reduces the amount of antibody required for a subsequent staining to as little as 10 µl. The complete process of cultivation, fixation, staining and imaging is done with ease using µ-Slides, µ-Dishes or µ-Plates. Detailed information about immunofluorescence protocols is given on pages 26-29.

... to high end imaging ...

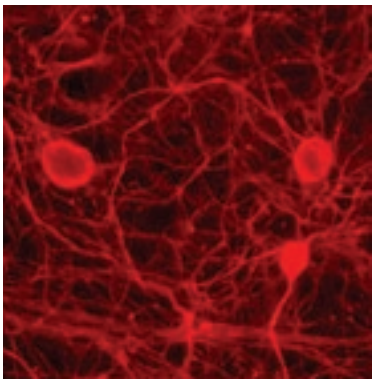
µ-Slides are valuable tools for monitoring molecular processes inside living or fixed cells. A foil like coverslip forms the bottom of all the µ-Slides. This bottom meets the optical requirements for various imaging techniques such as phase contrast, confocal, or two-photon microscopy.

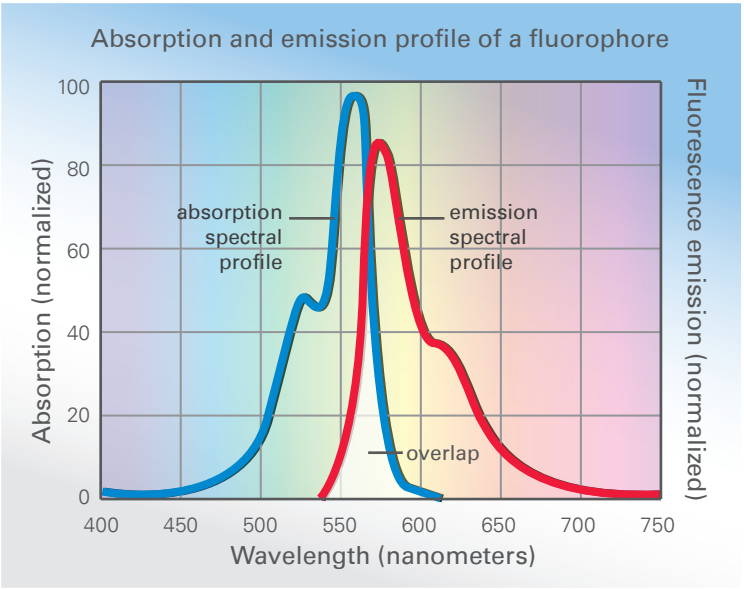
Today, the most common illumination method in biological research is fluorescence. Here, the specimen is illuminated with light of a specific wavelength which is absorbed. The fluorophore then emits light at a longer wavelength (of a different color than the absorbed light) as indicated in the graph below.

In order to meet the demands for TIRF and single photon measurements ibidi is now offering µ-Dishes with a glass surface. Detailed information about the optical requirements for the different imaging techniques are given on pages 54-55.

... and cell based assays

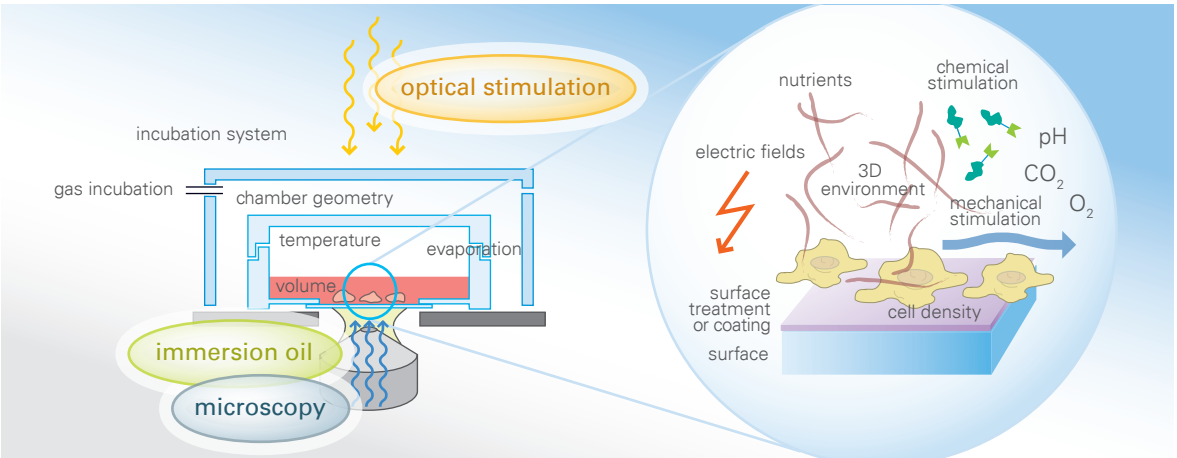
Time lapse systems (video microscopy) allow scientists to image multi cellular processes such as angiogenesis, chemotaxis, migration, and wound healing. For such defined applications specific µ-Slides have been developed. They enable these assays to be performed with precision and ease. The cellular behavior can be analyzed directly using non-invasive label-free methods with phase contrast. To view videos and to find protocols for these assays please visit the application section on the website www.ibidi.com.

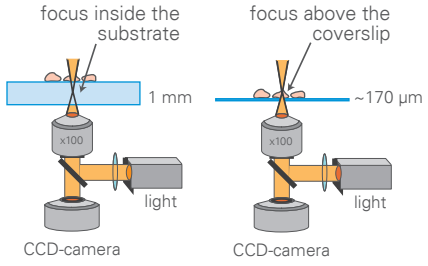




label	excitation (nm)	emission (nm)
DAPI	345	458
GFP	395	509
Alexa 488	494	519
Texas Red	589	615
YOYO-3	612	631

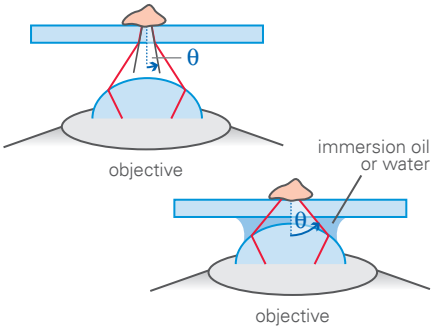
Optical properties – What do you need for high resolution microscopy?





No. 1.5 coverslip-like thickness

The thickness of the coverslip is a crucial aspect to imaging quality. The typical thickness for a coverslip is 0.17 mm (170 µm, No. 1.5). Most objective lenses for microscopy are corrected to this special thickness. Thinner or thicker substrates require correction collars on the objective lenses. That prevents formation of blurred images by spherical and chromatic aberrations.



Compatibility to immersion oil

Oil immersion is an important technique that is used to increase the resolution of the objective up to the physical limitation. Placing immersion oil instead of air between the objective lens and coverslip allows the amount of light collected by the lens to be significantly higher. This in turn increases the resolution and the signal to noise ratio in microscopic images.

Material	Refractive index
Air	1.0003
Water	1.33
Glycerol	1.47
Immersion oil	1.52
Glass	1.52
ibidi standard bottom	1.52

Standard refractive index

The refractive index *n* of a material describes how much the speed of light is reduced inside the material compared to absolute vacuum. The refractive index is often referred to as "optical density". For optimal microscope images the refractive index has to be 1.52.

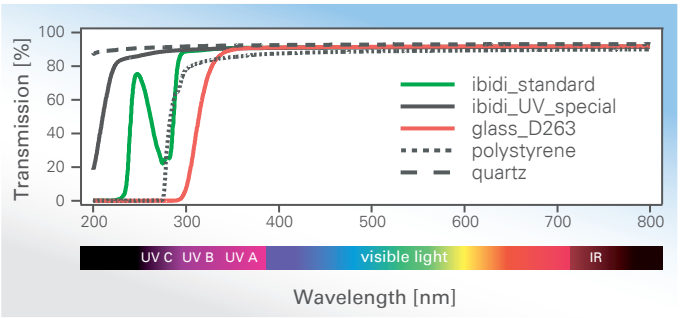
The numerical aperture NA of an objective lens summarizes the angles over which a lens can absorb light. NA is important as it describes the resolving power of a lens.

The numerical aperture is defined by **NA = n sin θ**

where *n* is the refractive index of the medium in which the lens works (e.g 1.52 for immersion oil), and *θ* is the half-angle of the maximum cone of light that can enter into the lens.

Transmission

Transmission is a crucial parameter for microscopy. It describes the property of a material in order to permit the passage of light at specific wavelengths. The more light is absorbed the less it can contribute to fluorescence excitation and image acquisition.

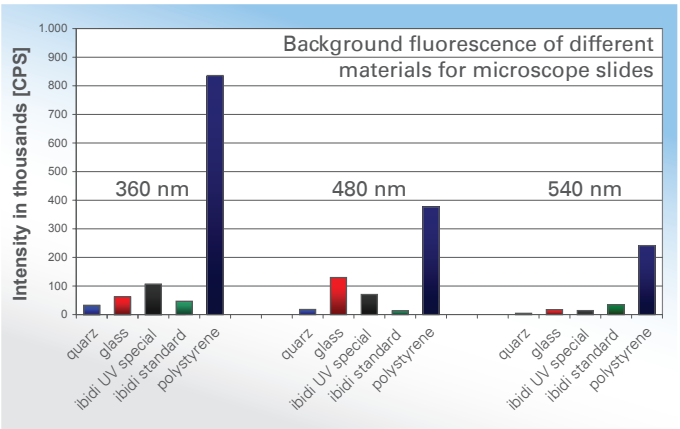


Material dispersion / Abbe number > 55

Material dispersion is defined as the variation of refractive index with wavelength. Therefore, the dispersion is a measure for chromatic aberrations. The Abbe number summarizes the dispersion into one value. It is calculated from the refractive indices at three different wavelengths. The higher the Abbe number, the better the optical quality for microscopy. A material with an Abbe number larger than 55 is considered to be well suited for high resolution microscopy. The ibidi standard bottom has an Abbe number of 56.

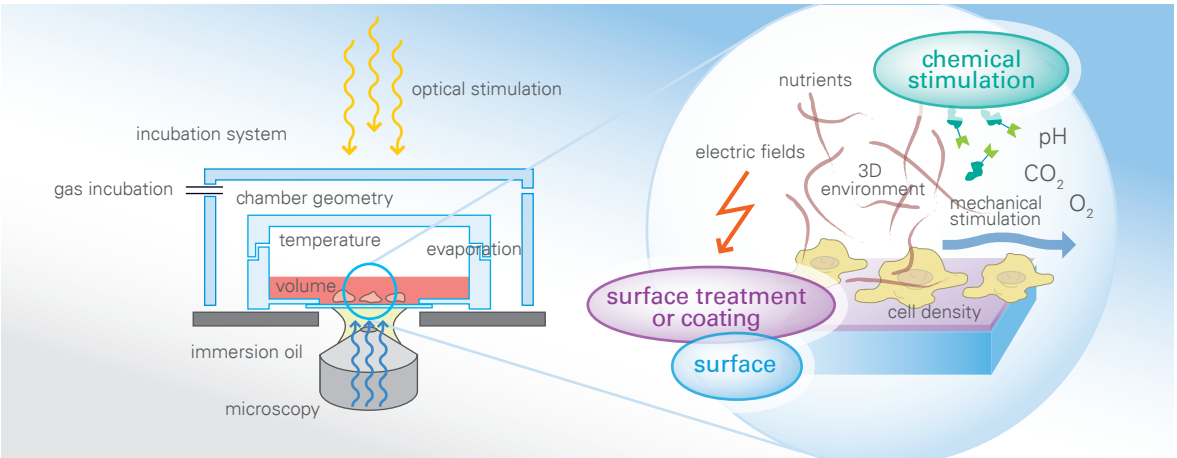
Low fluorescence background (autofluorescence)

Autofluorescence is a material property describing the intrinsic fluorescence without prior staining. The fluorescence signal comes from the material itself and contributes to the imaging process as noise. This can be annoying when trying to image faint fluorescence signals.



Material	Thickness	Refractive index (500 nm)	Auto-fluorescence	Abbe number
ibidi standard bottom	# 1.5	1.52	low	56
ibidi UV bottom	# 1.0	1.53	very low	58
glass coverslip	# 1.5	1.52	low	55
quarz glass coverslip	# 1.5	1.45	very low	67
polystyrene	-	1.56	high	33
PC	-	1.59	high	31
PMMA	#1.5	1.49	medium	57

Cell environment I – surfaces



The growth, development, and signaling of cultured cells strongly depends on the surface onto which the cells have been seeded. One of the advantages of the ibidi µ-Slides is that they can be treated similarly to standard plastic labware without compromising on the imaging quality.

ibidi standard surfaces

ibiTreat - tissue culture treated

ibiTreat is a physical surface modification for improved cell adhesion on µ-Slides. The surface is comparable to standard cell culture flasks and Petri dishes. The adhesion of cells to ibiTreat µ-Slides is strong enough to perform flow experiments simulating the physiological shear stress of the blood flow. This surface was tested with a vast range of different cell lines and primary cells.

hydrophobic, uncoated

The uncoated surface does not permit direct cell growth and can be used for your own specific coating procedures or for non-adherent cells, due to its hydrophobicity.

glass bottom

ibidi offers specialized products with a coverglass bottom using Schott glass D263. Glass bottoms are suitable for direct cell culture or special coatings. Glass can also be coated prior to cell seeding for adherent cells.

ibidi developed these glass surfaces especially for TIRF, single photon, and single molecule applications.

Coatings

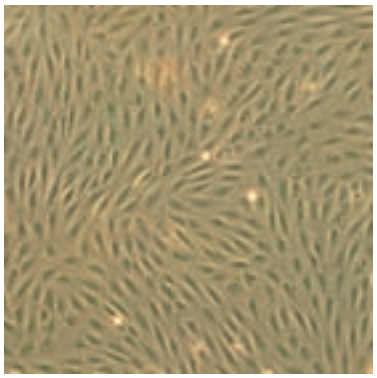
Collagen IV

Collagen IV is the primary type of collagen found in large, extracellular membrane structures and complex organs. Collagen IV substrates have been tested for a variety of standard cell lines such as epithelial, endothelial, nerve, and muscle cells. ibidi µ-Slides are coated with a mouse Collagen IV.

Poly-L-Lysine (PLL) / Poly-D-Lysine (PDL)

PLL / PDL is a polymer of the amino acid L / D-Lysine. This polymer is one of the most commonly used adhesion substrates. It is suitable for a large variety of cell types, especially for neuronal cultures. Adhesion via this polymer is mediated by an integrin-independent mechanism.

PDL-coated µ-Slides are available on demand only. Delivery time is estimated at three weeks.



RAT1 on Collagen IV

Fibronectin

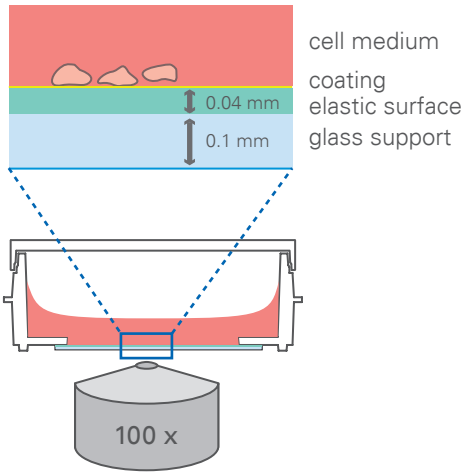
Fibronectin is a glycoprotein widely used in cell culture coatings. It plays an important role in cell surface interactions, which are mediated by the RGD motive. It allows neurite outgrowth and has been used for glial and neural cells. ibidi µ-Slides are coated with a human Fibronectin.

Due to its short shelf life, Fibronectin coated µ-Slides are available on demand only. Delivery time is estimated at three weeks.

ibidi special surfaces

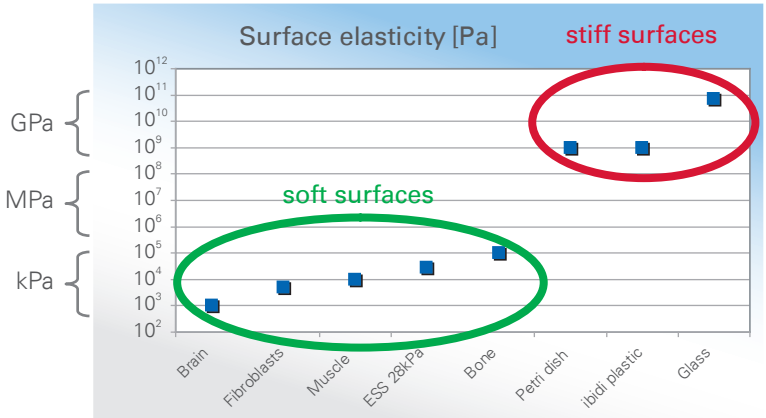
ESS, elastically supported surface, 28 kPa

Cells in tissue grow under different conditions as commonly found in *in vitro* cell cultures. A crucial parameter that effects cell proliferation, differentiation, and overall function is the surface stiffness/elasticity. Recent research has shown significant evidence that this physical parameter must be taken into consideration. The elasticity (Young's Modulus) of most plastics used for cell culture is around 1 GPa. Young's Modulus of glass is approximately 70 GPa. In contrast to plastic and glass, the Young's Modulus of mammalian cells (i.e. muscle cells) is below 100 kPa. Therefore, the natural cell environment is at least 100,000 times more elastic.



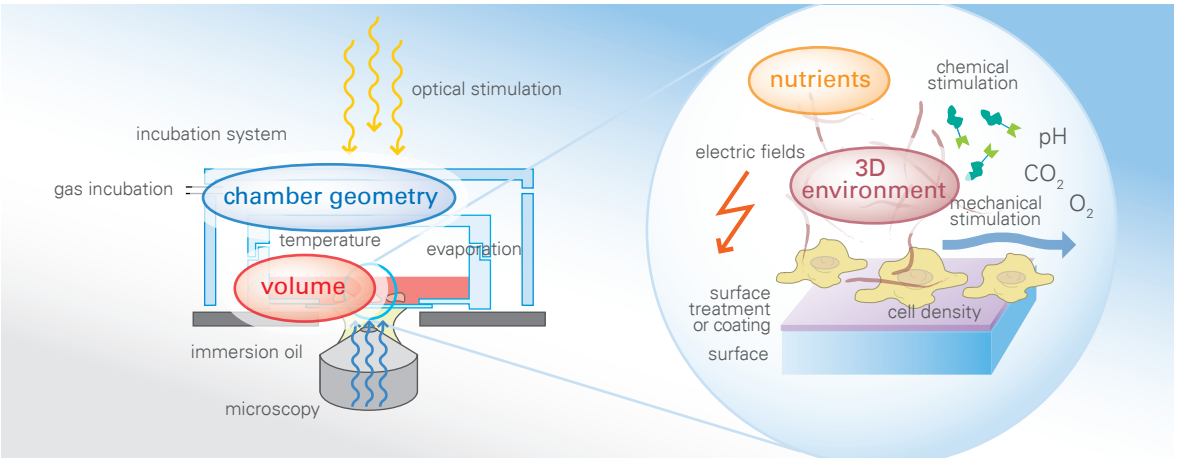
ibidi's elastically supported surface ESS 28 KPa is comparable to cells and tissue elasticities.

The uncoated ESS surface is hydrophobic and allows no direct cell growth. For convenient cell growth we recommend specific ECM coatings, like Collagen, or Fibronectin.



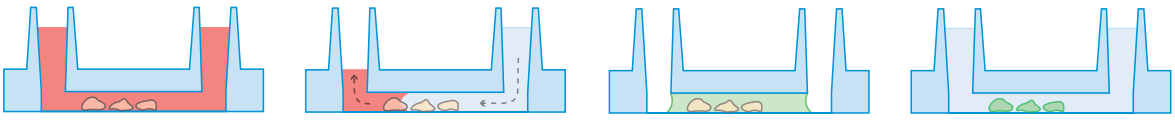
Please visit www.ibidi.com for detailed coating protocols.

Cell environment II – geometry

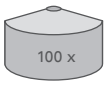


all-in-one chambers

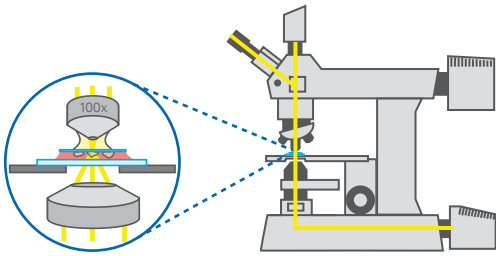
1) cultivation 2) fixation 3) staining 4) imaging



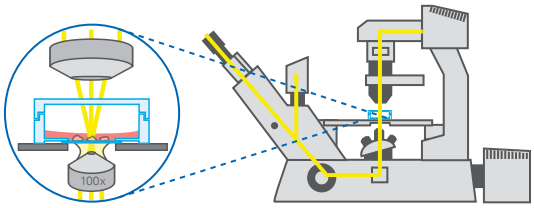
ibidi µ-Slides, µ-Dishes, and µ-Plates are designed as all-in-one chambers.



upright microscope



inverted microscope



Compatibility with ibidi products:

- µ-Slide upright [0.7]
- µ-Chamber 12 well

- all other ibidi µ-Slides, µ-Dishes, and µ-Plates
- ibidi heating system

In cell biology upright microscopes are used for:

- samples squeezed between slide and coverslip.
- fixed samples like cells and tissue sections.

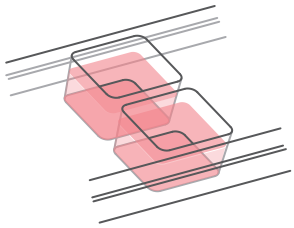
Not recommended for live cell imaging today.

Inverted microscopes are popular in live cell imaging because:

- cells sink to the bottom and onto the coverslip for adherence.
- no contact between objective and sample. Sterile work is possible.
- exchange of liquids is possible due to sample access from the top.
- access to sample with micropipettes.

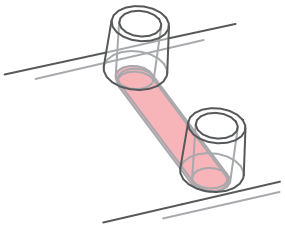
Geometry

open well format



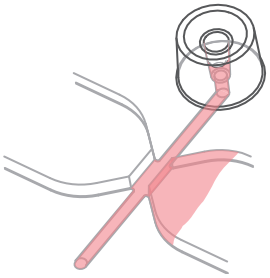
- common formats
- easy handling
- large volume

cell culture channel



- excellent imaging
- easy liquid exchange
- low volume

refined geometry



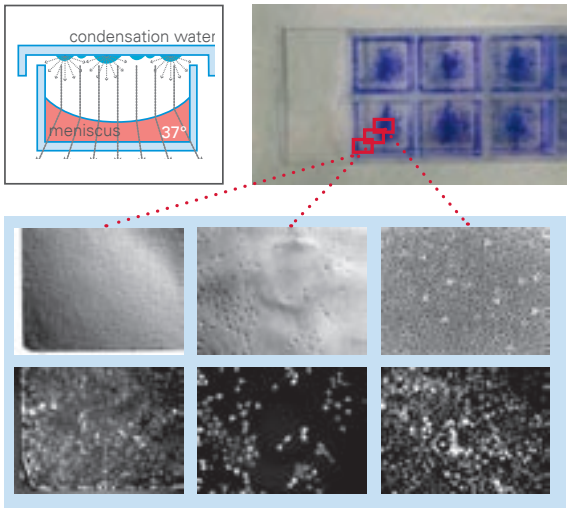
- designed for special purposes, e.g. gradients, gel matrices

Homogeneous distribution of cells

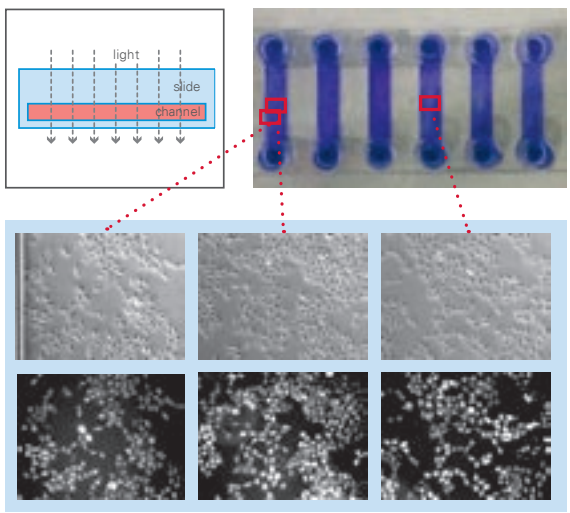
To demonstrate the influence of the slide geometry, cells were cultivated in various formats. Cell layers were visualized macroscopically by crystal violet staining and additionally by fluorescence and phase contrast microscopy.

The macroscopic photographs show that cells cultured in open wells form characteristic patterns. One common pattern found, is that some cells congregate to the edges of the well. Attached to that area is normally an area with fewer cells, while in the middle of the well the cell density reaches its maximum. In contrast, when cultured in a channel format (such as µ-Slide V) the cell distribution always appears homogeneous. These macroscopically derived results were confirmed by phase contrast and fluorescence microscopy as shown.

Cell densities in open wells are very dependent on handling during cell seeding. Unlike in open wells, the cell densities in the channels neither vary with the position inside the slide, nor with the handling and treatment during and after the cell seeding.

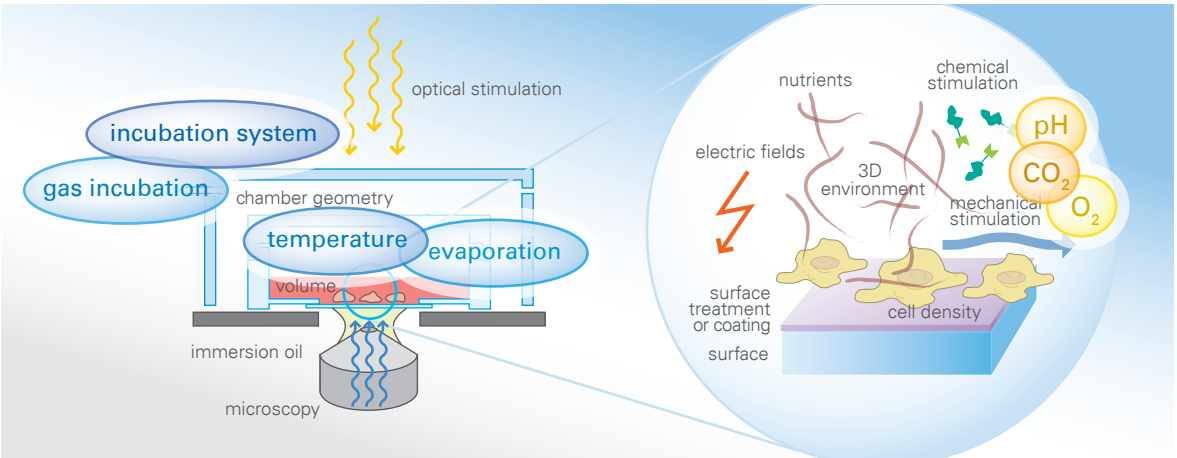


cell distribution in open wells



cell distribution in cell culture channels

Live cell imaging – *in vivo* conditions on the microscope



Temperature stability

Cells only behave naturally when cultured at the temperature of their biological environment. Some mammalian cells will only survive in a narrow temperature range around 37°C, while others will be more stable at lower temperatures. With this observation one may suggest that metabolism is influenced by temperature. Therefore observed parameters like proliferation, adhesion, protein expression and others will be dependent on temperature. Results observed at different temperatures should not be considered as comparable findings.

Focus stability

In order to maintain focus stability, the temperature has to be stable. The requirements for focus stability strongly depend on the numerical aperture of the objective and on the technique used. For example, a 100 x objective with NA = 1.45 will sense focus drift even below 0.5 µm, as would TIRF microscopy and confocal microscopy. However, when using a 10 x objective in phase contrast, a focus drift of a few µm is not crucial. In general, optical stability better than 1 µm is easiest achieved using glass coverslips (which we recommend to use for extreme magnification and TIRF). In the case of high resolution time lapse microscopy, we recommend the application of an electronic focus system like the Nikon Perfect Focus System (PFS) or auto focus devices.

CO_2 concentration

By far the most important buffer for maintaining acid-base balance in the blood of higher mammals is the carbonic-acid-bicarbonate buffer. The simultaneous equilibrium reactions of interest are

$$H^+ + HCO_3^- \leftrightarrows H_2CO_3 \leftrightarrows H_2O + CO_2$$

The CO_2 concentration in exhaled air is 4% to 5% indicating that the CO_2 dissolved in blood is in equilibrium with air containing this CO_2 concentration. Bicarbonate buffered culture media are designed to exhibit a pH of 7.4 at a CO_2 concentration of 5% in ambient air.

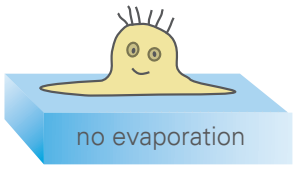
Using bicarbonate buffered media the CO_2 concentration in the ambient air of 5% is a common standard in cell culture.

Humidity and evaporation

Salt and protein concentrations are crucial for natural cell behavior. In order to keep costs at a reasonable level, vessel sizes of standard culture labware are in the range of µl to ml, with a trend of becoming smaller. Evaporation increases concentrations of substances in cell cultures in an undefined way. Minimization of evaporation can be obtained by controlling the humidity of ambient air to nearly 100%. Average values reached in real incubators are 80-90%. The lids of all ibidi products are designed to minimize evaporation. ibidi also provides a few products which are sealed against evaporation, such as the µ-Dishes and µ-Slide Chemotaxis.

Note: All ibidi gas incubation systems provide active feedback controlled humidity regulation.

changing concentration

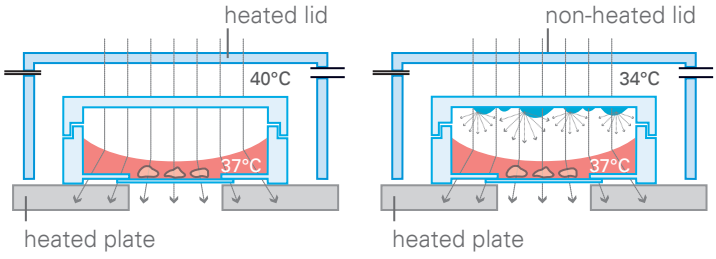


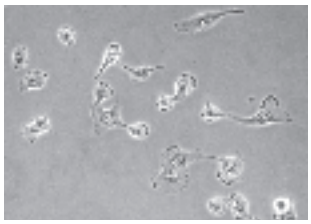
constant concentration

Condensation

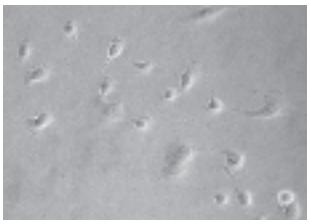
Humidity in ambient air can lead to condensation on all surfaces. If these surfaces are in the optical pathway, small water droplets lead to light scattering. This diminishes the optical quality of transmitted light microscopy, i.e. phase contrast and DIC.

The ibidi heating system solves this problem by using a separately controlled heated glass lid that covers the stage top incubator.





phase contrast without condensation



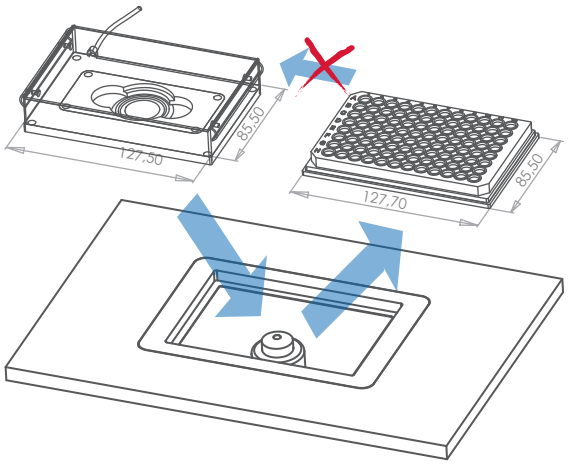
phase contrast with condensation

Compatibility to inverted microscopes

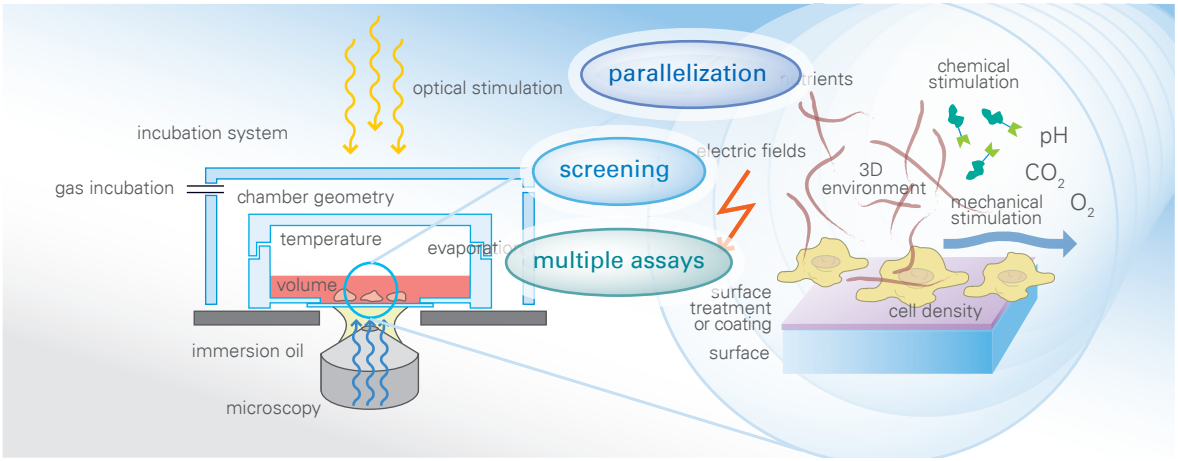
The ibidi heating system fits on every inverted microscope provided that the stage has a holder for multi-well plates. This is a standard format found on microscopes.

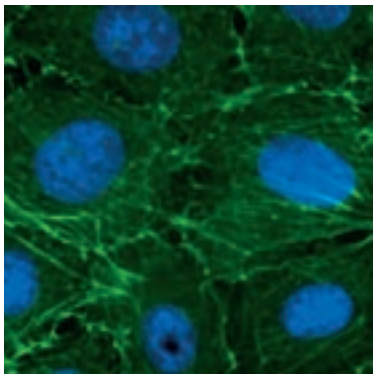
The small stage top incubator is simply inserted in the multi-well plate holder. Its compact design allows the heated stage and incubation unit to be easily moved from one microscope to another.

Please note that the outer dimensions of the heated stage have a multi-well format. Therefore, it cannot host multi-well plates.

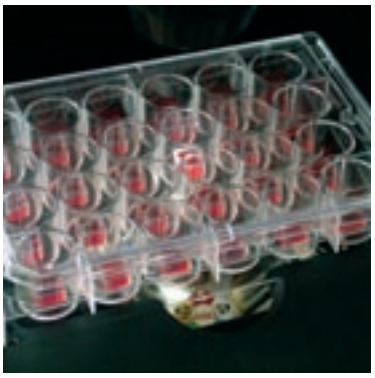


Screening



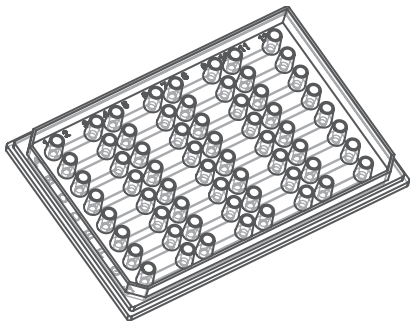


Over the last decade, automation turned microscopy into a routine screening technique. Screening equipment ranges from simple motorized microscopes and computer controlled stages to fully automated imaging systems including robotics and cell culture incubation environments. Today's high performance computers allow rapid high content image processing as well as storage of large amounts of data. Screening in microscopy is applied to investigate structure formation as in tube formation assays, morphological changes as in live dead assays, or molecular interaction and protein (co-)localization via fluorescence.



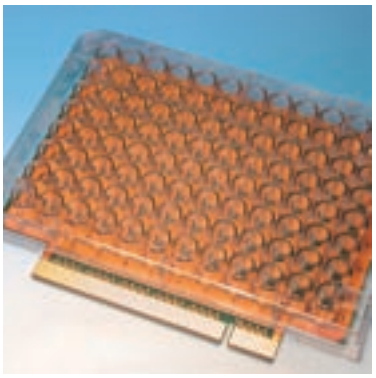
Multi-well plates

Multi-well plates have been used in high throughput screening for many years. Standard multi-well plates have a 1 mm thick bottom and are made of polystyrene. These plates are neither suited for high resolution nor fluorescence microscopy.



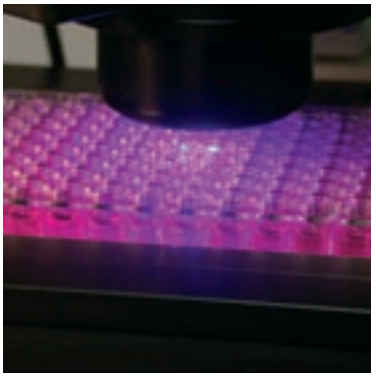
The ibidi technology provides all important features for enhanced microscopy: a coverslip like bottom of standard No. 1.5 thickness with extremely low autofluorescence in combination with the unique tissue culture treatment ibiTreat. Through the µ-Slides, µ-Dishes, and µ-Plates, the ibidi technology has proven to be biocompatible with immortalized cell lines as well as many primary cells. Another remarkable feature is the excellent stability against all common solvents used in cell based assays.

High content screening also answers the demands of systems biology, requiring multiple parameters to be measured from one single cell experiment. Besides optical techniques, screening can also be performed in a non-invasive way via the impedance based ECIS technology (see pages 104 - 105).



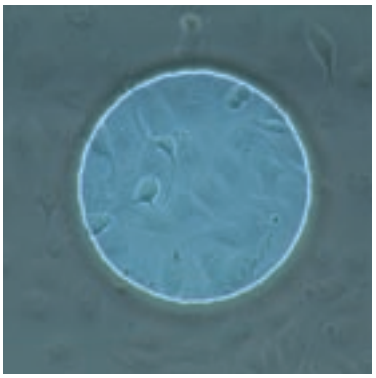
ibidi develops solutions for multi-well plates ranging from 6 wells up to 384 wells. The production process is scalable even in case of larger well numbers.

Unlike plates with glass bottoms glued to bottomless polystyrene plates, the ibidi bonding technique connects the bottom to the raw plate avoiding the use of any glue. It is therefore independent from the complexity of the top part such as the well number and size of micro structures.

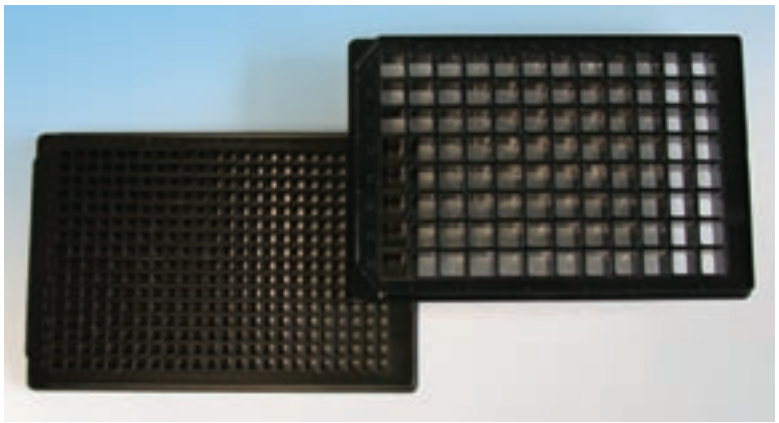


Customer specific screening solutions

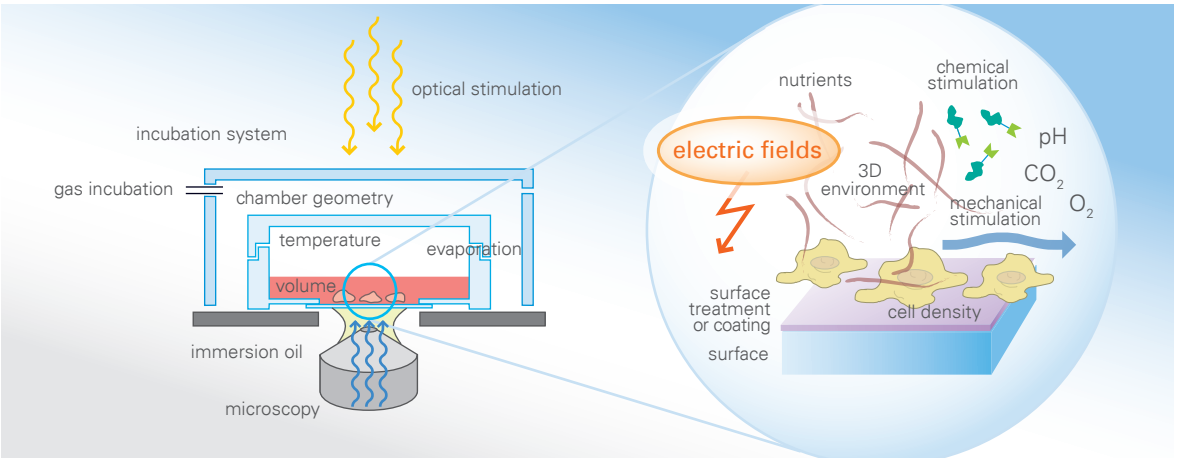
In addition to the regular product range, ibidi offers OEM solutions for custom-specific screening consumables. We can adapt any consumable to the needs of your high content and high throughput screening instrumentation as well as to the requirements of the specific cell culture and test environment.



There is almost no restriction in possible designs of screening plates for specific applications. Besides multi-well plates we also recommend taking micro-channels into account when thinking about small reagent volumes and cell numbers. In closed geometries evaporation is minimized while optical quality is improved. Only in micro channels phase contrast can be applied on sample volumes of several micro liters.



Cells in electric fields



Electric fields can be used for both, external stimulation and monitoring of intrinsic electric parameters of cells. For example, stimulation in muscle cells like cardiomyocytes leads to better simulation of *in vivo* conditions. A label free cell analysis technique is Electric Cell-substrate Impedance Sensing (ECIS). As cells attach and spread upon electrodes, their insulating membranes block and constrain the current flow. By monitoring the resulting variations of the impedance this technology allows measuring parameters such as proliferation, and migration.

Applications of planar electrodes

- application of defined voltage and electrical fields / current to cells, cell clusters, and tissue
- observation and stimulation of cells in electric fields (AC or DC)
- measurement of voltage, field strength, current, and resistance/impedance of cell surfaces and entire cell monolayers

Design of planar electrodes

Non-insulated conductive tracks

Non-insulated tracks can be used for a broad range of applications including

- applying electric fields onto cells
- electric stimulation e.g of cardiomyocytes
- dielectric focusing
- electrotaxis of cells
- polarization of cells

Insulated conductive tracks with “large” electrodes

Large electrodes connected by insulated tracks are typically used for the analysis of two-dimensional cell cultures. Usually, the impedance is measured on electrodes with diameters of approx. 250 µm. The ECIS technology is the most prominent example of these kinds of planar electrodes.

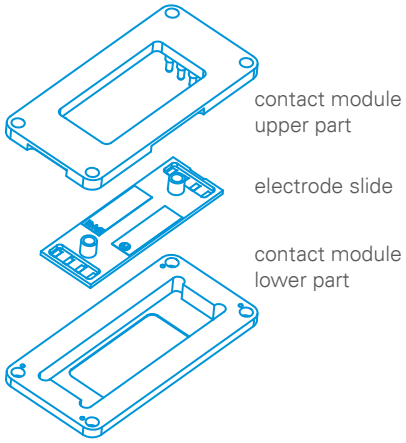
Insulated conductive tracks with “small” electrodes

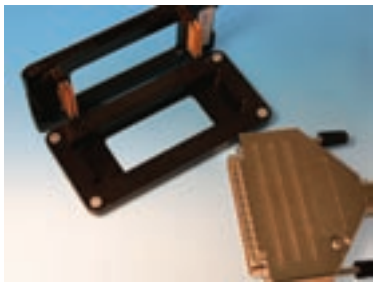
Small electrodes are widely used for single cell measurements in neuronal or cardiac research. The small electrodes of approx. 20 µm are often assembled to MEAs (multi electrode arrays).

ibidi electrode slide system – consists of the contact module including an electric connecting interface and the electrode slides

Contact module

The contact module provides an easy to handle electric interface on a microscope stage



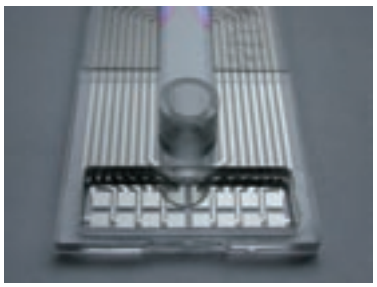


Contact module adapted to ibidi standard heating system

ibidi electrode slides*

The ibidi electrode slides are developed for the microscopic observation, external stimulation and/or analysis of intrinsic electric parameters on living cells. Our goal is to provide you with a flexible electrode layout design. Please contact us with your special requirements.

Product details are given on page 96.



Microscopy slide with integrated electrodes

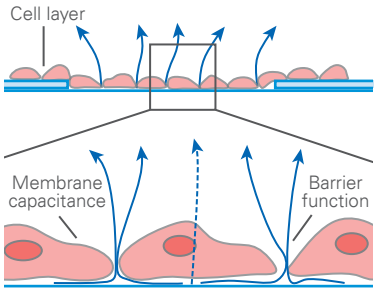
Features and characteristics

- possible electrode size: > 25 µm
- possible geometry: any 2D geometry
- number of independent electrodes: 32
- max. number of contacts in contact module: 32
- electrode materials: gold or titanium
- support material: glass or ibidi standard bottom
- mode: (AC or DC)
- supported µ-Slides: µ-Slide I Luer
  Please contact us if you wish to use other formats.

Customized electrode layouts possible

Electric Cell-substrate Impedance Sensing (ECIS)

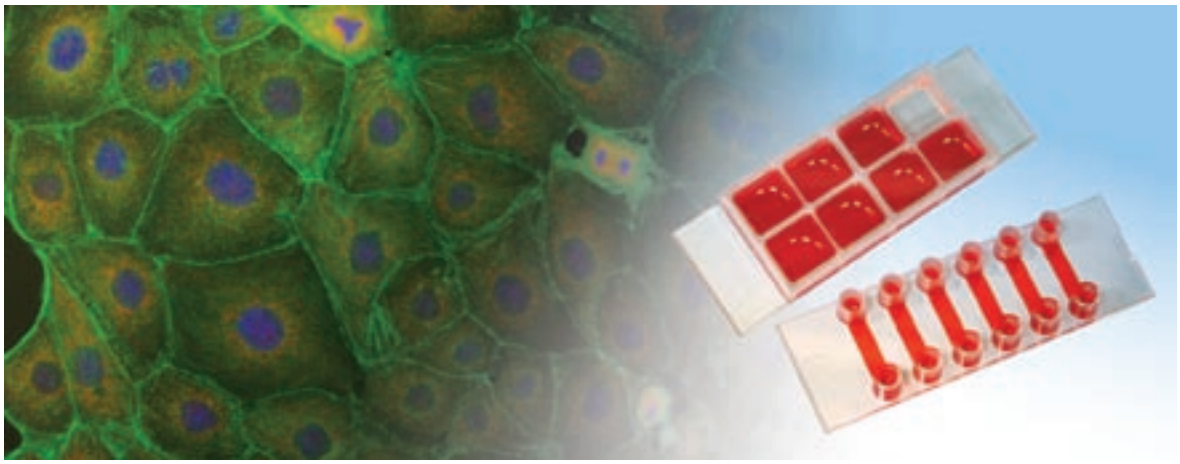
ECIS refers to a non-invasive approach of monitoring living cells in vitro. The cells grow on the surface of small and planar gold-film electrodes, which are deposited at the bottom of a cell culture dish. In confluent cell layers the measured impedance is mainly determined by the shape of the cells. If cell shape changes occur, the current pathways through and around the cell bodies change as well, leading to a corresponding increase or decrease in impedance. Thus, by recording time-resolved impedance measurements, cell shape changes can be followed in real-time.



Find more information on page 104

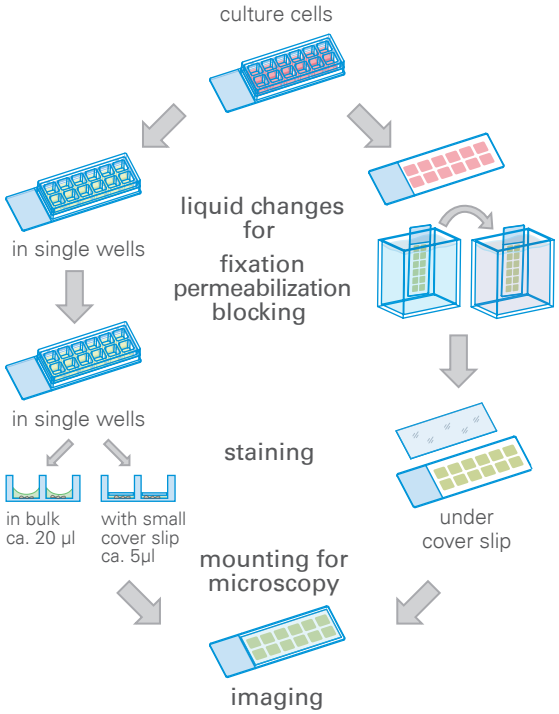
** developed in cooperation with NMI, Natural and Medical Sciences Institute at the University of Tuebingen*

Immunofluorescence



Immunofluorescence techniques are widely used in cell biology and tissue samples. Every fluorescence protocol consists of four major steps: cultivation, fixation, staining, and subsequent imaging of the cells. Unlike most plastic materials for cell culture, the ibidi µ-Slides and µ-Dishes are compatible with alcohols, acetone, acids, and para-formaldehyde.

Different protocols possible



Immunofluorescence in the removable µ-Chamber 12 well

ibidi now provides the easiest and most convenient solution for removable culture wells. It consists of a silicone gasket with 12 chambers for individual cell culture and incubation conditions. The silicon piece is easily removed by hand and leaves no residue on the glass to which it was attached. This also allows for the easy and reliable mounting of coverslips. This technique is similar to the classical method, but µ-Chamber 12 well comes ready to use in a sterile package and the culture volume is decreased to only 250 µl.

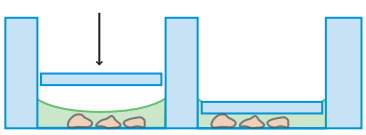
Advantages

- sterile and ready to use
- 12 parallel tests on one slide

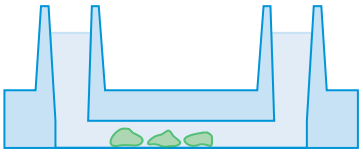
Limitations

- large volume for cultivation and staining

Open slides vs. channel slides

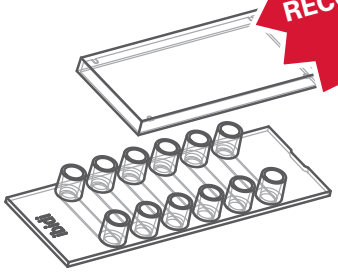


In open formats a better imaging quality can be achieved by using small coverslips to prevent meniscus formation. Such a "lid" is integrated intrinsically in channel structures.

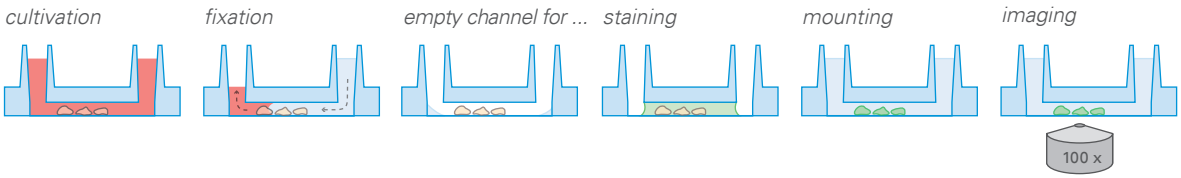


Immunofluorescence in channel formats

MOST RECOMMENDED



The ibidi channel µ-Slides are ideal to exchange liquids as media, buffer, washing, or staining solutions. As long as there is a difference in the filling level between one reservoir and the other, liquids will flow smoothly through the channel making subsequent washing steps as easy as possible. As the channel geometries are small, the amount of staining solutions such as antibodies is reduced to as little as 30 µl (µ-Slide VI 0.4).

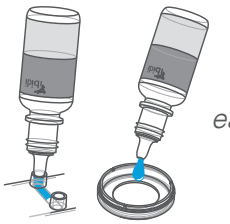


Advantages

- exact liquid exchange
- fast protocol
- easy and rapid handling
- sterile and ready to use
- 30 µl for staining only
- no cell transfer
- no coverslip handling

Limitation

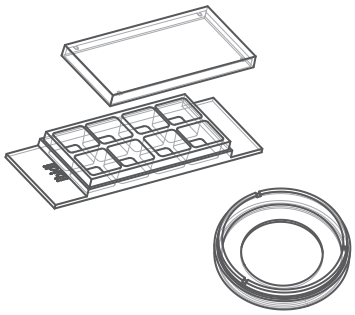
- storage time is restricted to several months



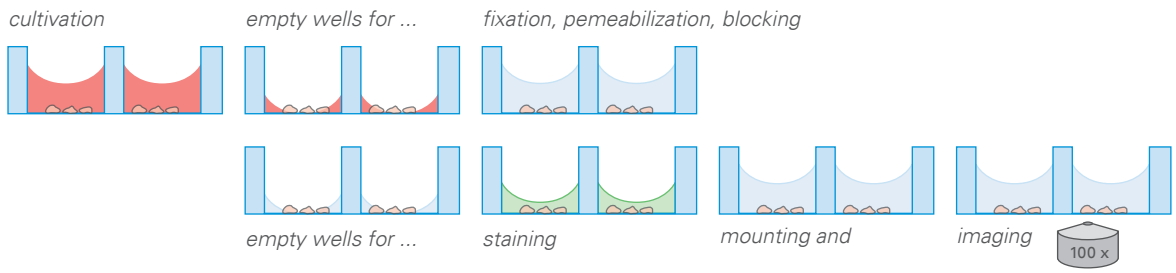
easy channel / well filling

ibidi Mounting Medium has been especially developed for the use with µ-Slides.

Immunofluorescence in open formats



The ibidi µ-Slide 8 well and the µ-Dish allow the use of standard immunofluorescence protocols in an all-in-one chamber. Here, cells are cultured and incubated in the classic open well geometry. In these wells fixation, permeabilization, and staining steps are also done. Finally, the sample can be observed through the coverslip-like bottom using high resolution microscopy.



Advantages

- easy and rapid handling
- sterile and ready to use
- no cell transfer
- no coverslip handling

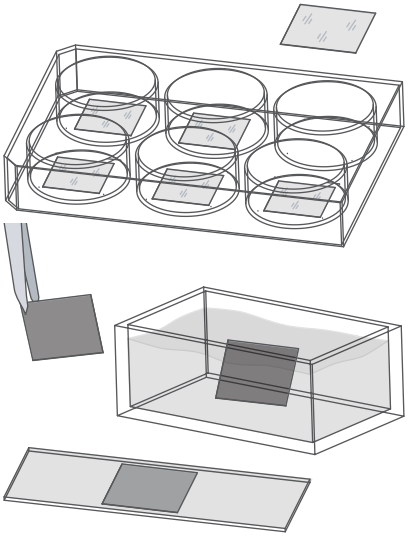
Limitations

- storage time is restricted to several months
- higher amount of staining solution

Immunofluorescence

The protocol listed below compares the ibidi method for performing immunofluorescence assays to the classical method. The main advantages are faster preparation and simpler parallelization. In most cases it is recommended to use channel slides for immunofluorescence. If it is necessary to keep samples for several years, the µ-Chamber 12 well is offered. Its removable chambers allow mounting a coverslip using the well known nail polish technique.

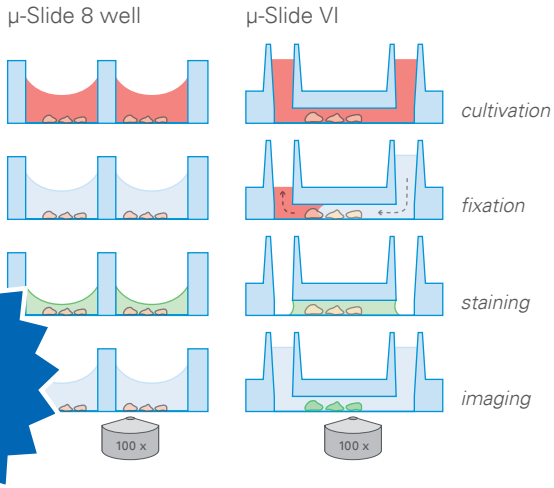
TIME CONSUMING AND TEDIOUS



Mounting cells on coverslips
(Old method with nail polish mounting)

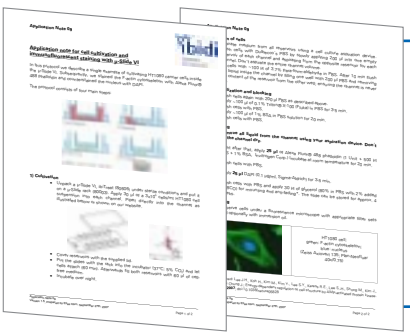
- sterilize coverslips and slides
- coat the coverslips
- put sterile coverslips into 6-well plate
- seed cells in large volume
- peel the coverslip out
- wash
- fix cells
- wash
- stain cells
- wash
- mount cells with mounting medium
- mount coverslip with nail polish

FAST AND EASY



Mounting cells with ibidi µ-Slides
(New method using all-in-one chambers)

- sterilize coverslips and slides
- coat the coverslips
- put sterile coverslips into 6-well plate
- seed cells in large volume
- peel the coverslip out
- wash
- fix cells
- wash
- stain cells
- wash
- mount cells with mounting medium
- mount coverslip with nail polish

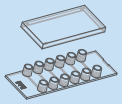
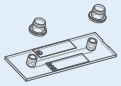
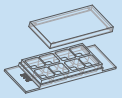
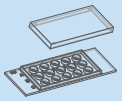
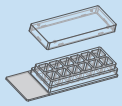


Detailed fluorescence staining protocols

Find detailed protocols on how to do a staining in the application section of our website: **www.ibidi.com**

There, all steps from the initial cell seeding, the staining, fixation and the final mounting are shown in detail.

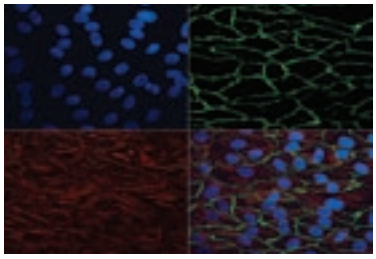
All ibidi slides and dishes are designed for immunofluorescence assays, with each format having a different method of preparation. For example, the µ-Slide VI 0.4 is typically used to culture and incubate cells with reagents for general immunofluorescence assays. More complex physiological assays using immunofluorescence, such as chemotaxis, tube formation, and long term cultures under flow conditions would utilize the µ-Slide Chemotaxis, µ-Slide Angiogenesis or µ-Slide VI 0.1

	Channel-Slides – all-in-one			Open chambers – all-in-one			Removable chambers	
	 µ-Slide VI 0.4	 µ-Slide VI 0.1	 µ-Slide I 0.2a Luer	 µ-Slide 8 well	 µ-Dish 35 mm high	 µ-Slide Angiogenesis	 Culture-Insert StemCell	 µ-Chamber 12 well
Volume staining reagent per well or channel	20 µl	2 µl	50 µl	100 µl	400 µl [1] 20 µl [2]	20 µl	5 µl	100 µl [3] 10 µl [4] 40 µl [5]
Growth area per well or channel	0.6 cm²	0.17 cm²	2.5 cm²	1.1 cm²	3.5 cm²	0.13 cm²	0.03 cm²	0.56 cm²
Culture volume	150 µl	120 µl	170 µl	300 µl	2 ml	50 µl	10 µl [6]	250 µl
Number of independent tests	6	6	1	8	1	15	4	12
Application examples (IF = Immunofluorescence)	IF after cell culture and incubation	IF with low cell numbers and flow experiments	IF at low density cultures and flow experiments	IF after cell culture and incubation	IF after cell culture and incubation	IF in small volumes with standard geometry	IF in very small volumes after culture and incubation	IF and long term storage of samples
All-in-one chamber	yes	yes	yes	yes	yes	yes	no	no

1) if staining is done just by filling the volume
2) if a coverslip is used for incubation in the inner well
3) if staining is done in the reservoirs
4) if staining is done with small coverslips
5) if staining is done with one coverslip over the full slide area
6) The volume can be increased placing the insert into any culture well.

For immunofluorescence protocols please visit www.ibidi.com

Application examples



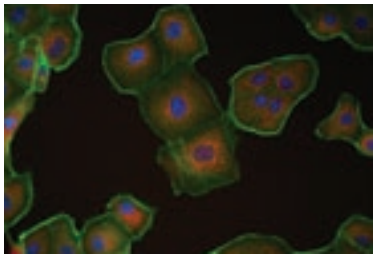
Human umbilical vein endothelial cells (HUVEC) cultured under flow conditions in µ-Slide I 0.4 Luer

blue: nucleus (DAPI)

green: VE-caherins (Alexa 488 conjugated antibody)

red: actin cytoskeleton (Cy5 conjugated antibody)

(courtesy S. Zahler, Munich, Germany)



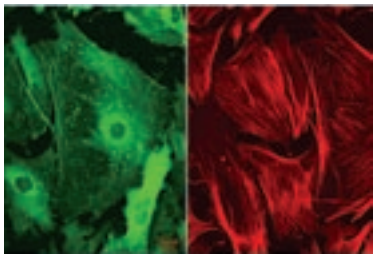
Cell line Madin-Darby canine kidney (MDCK) cultured in µ-Slide VI 0.4

blue: nucleus (DAPI)

green: actin cytoskeleton (Alexa 488 conjugated antibody)

red: mitochondria (MitoTracker)

(courtesy ibidi research group)



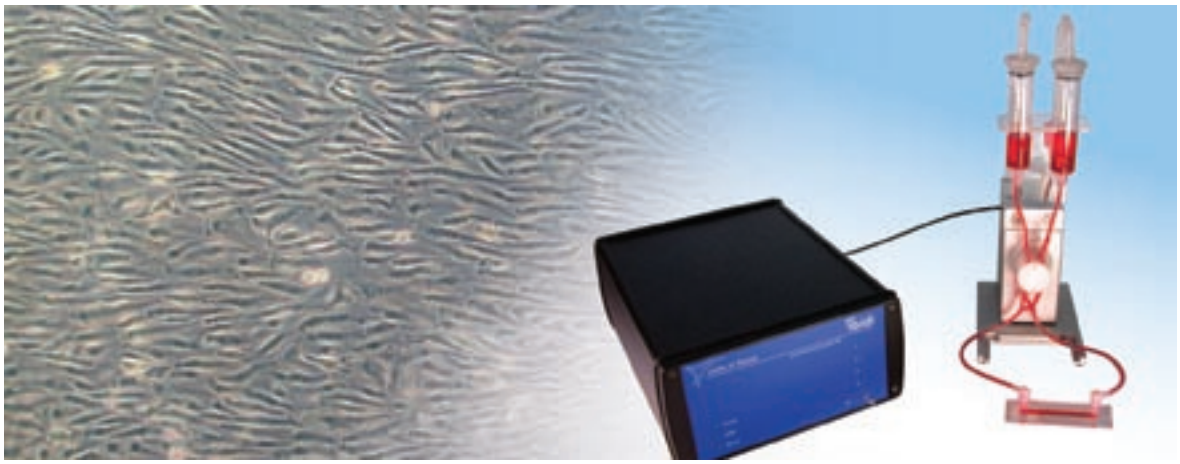
Differentiated mouse fibroblasts cultured on elastic surface (ESS 28 kPa)

green: zyxin (Alexa 488 conjugated antibody)

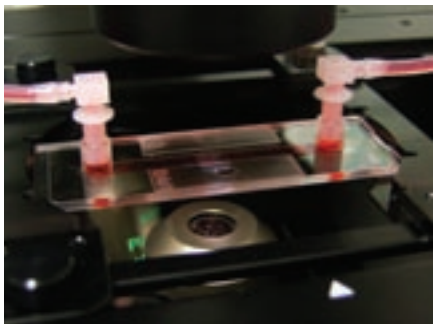
red: alpha- smooth muscle actin (Cy5 conjugated antibody)

(courtesy R. Merkel, Jülich, Germany)

Cell based perfusion assays



In vivo, several adherent cell types are exposed to mechanical shear stress such as is found in blood vessels. This mechanical stimulus has a great impact on the physiological behavior and adhesion properties of cells.



ibidi consumables are tested for both, static and perfusion cell cultures. Many of the µ-Slides were especially designed for performing flow assays. Also being compatible with any inverted microscopy technique they are ideally prepared for parallel perfusion assays directly on the microscope or inside the incubator.

Depending on the experimental requirements, peristaltic pumps, syringe pumps, or even simple gravity flow can be used. For various perfusion protocols please visit page 33 or contact ibidi for personal consulting.

Vessel	shear stress dyne / cm²	
	average	maximal
Aorta	-	> 15
Artery	-	5 - 10
Middle vein	0.8 - 1	-
Small vein	0.4	-

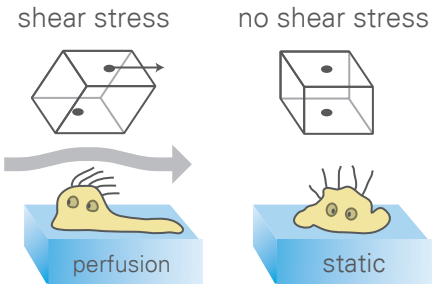
Experimental end points using µ-Slides

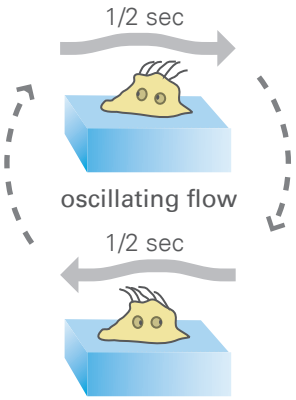
- real-time monitoring of morphological changes in living cells
- protein localization using fluorescence
- profiling secreted proteins in conditioned media
- cell adhesion
- gene expression profiling after cell detachment

Basic questions for setting up a flow experiment

In order to set up the right experiment you should first answer the following questions:

1) What kind of experiment is planned: Adhesion assay or cell culture under flow conditions?
2) What shear stress or shear rate will be applied?
3) How long will the experiment take?
4) Should it be a one way setup, or a circular setup?
5) Are the reagents used expensive or cheap and available in large amounts?
6) Should the experiment be done at 37°C and at 5% CO_2 atmosphere?
7) What other aspects are important?





Shear stress in general

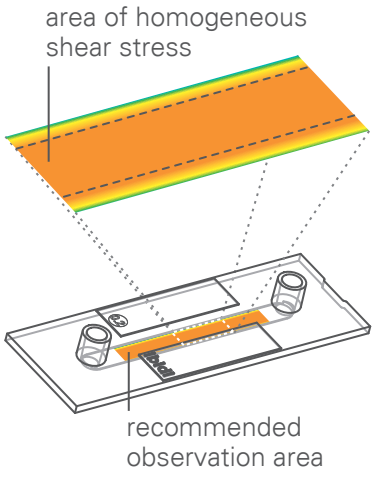
... is the mechanical force induced by friction of a liquid to the distal cell membrane. Cells might be able to counteract deformations caused by shear stress by rearranging their cytoskeleton. Other shear stress dependent effects are changes in metabolism, gene expression, and differentiation. Physiological shear stress values vary from 0.4 dyne/cm² (small veins) to 20 dyne/cm² (peak flow in abdominal aorta).

Turbulent flow

... near surfaces is characterized by changes in flow rate and direction. For simulating turbulent flow it is accepted to use laminar flow of oscillating direction within half a second. This is because turbulent flow cannot be achieved in micro channels at physiological flow regimes for physical reasons.

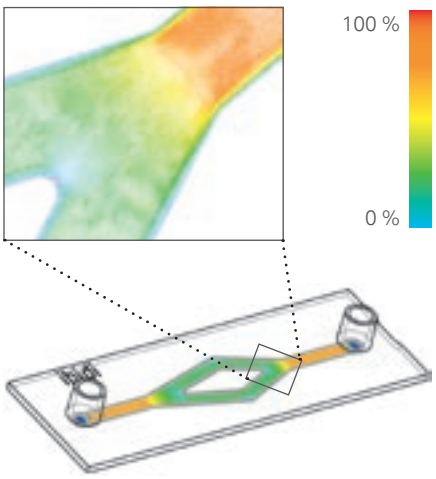
Laminar shear stress

... is expected in most healthy biological vessels like arteries and veins. Experimentally it is achieved by perfusing medium through microchannels of low heights. The observation area of homogeneous laminar shear stress includes the whole channel area without small stripes close to the side walls and near the reservoirs (both in the range of the channel height). For homogeneous laminar shear stress experiments µ-Slide I Luer, µ-Slide VI, and µ-Slide III Luer are recommended.



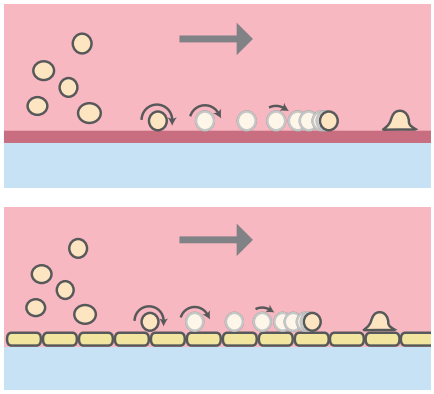
Non-uniform, laminar shear stress

... occurs at branching sites and other obstacles of vessels *in vivo*. Experimentally it can be achieved by gradually varying flow rates. It can be used for investigating cells at different shear stresses in one sample. If performing more advanced assays, it can be used for studying cells and their communication at positions with strongly varying shear stress. The µ-Slide y-shaped was designed for studies of non-uniform shear stress. In the branched region the prevalent shear stress is approximately half of that found in the single channel design. At the branching point experimentators should refer to our numerical simulations for exact shear stress values. Please visit www.ibidi.com for Application Note 18.



Detailed product information on pages 73 and 79

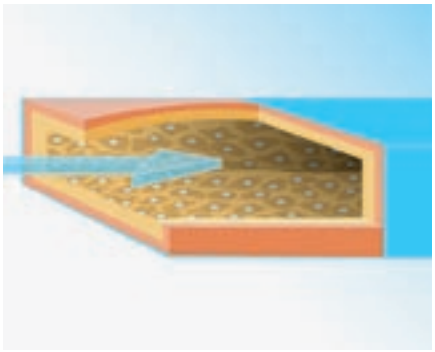
Cell based perfusion assays



perfusion assays on various supports

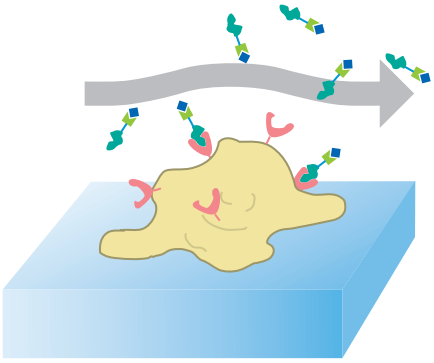
Adhesion assay

Application: blood cells to protein surfaces
Rolling and adhesion of suspended cells such as platelets, leukocytes, monocytes on substrates like adhesion proteins or confluent cell monolayers
Flow characteristics: continuous, laminar
Recommended pumps: syringe pumps, ibidi pump system, peristaltic pump
Duration: 20 min - 2 hours
Recommended slides: µ-Slide VI, µ-Slide I Luer, µ-Slide III
Experimental environment: room temperature or incubation conditions*



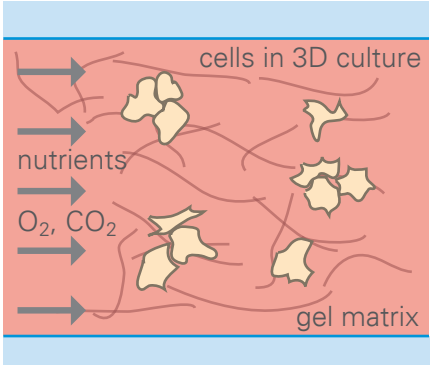
Cell culture under shear stress

Application: endothelial cells under flow
Influence of shear stress to endothelial cells, preparing cells while mimicking *in vivo* perfusion conditions, antibody stainings, formation of plaques on endothelium, biofilm formation of microorganisms
Flow characteristics: continuous, laminar, non uniform, oscillating for turbulence simulation
Recommended pumps: ibidi pump system
Typical duration: 12 hours up to several weeks
Recommended slides: µ-Slide VI, µ-Slide I Luer, µ-Slide y-shaped
Experimental environment: incubation conditions*



Stop flow experiments

Application: Ca^{2+}-imaging
Defined medium exchange for optimal feeding, online drug delivery, live stainings
Flow characteristics: laminar, short periods of flow in between non flow phases
Recommended pumps: manual liquid delivery (pipette, gravity flow, syringe etc.), syringe pumps, peristaltic pump
Recommended slides: µ-Slide VI, µ-Slide I Luer, µ-Slide I



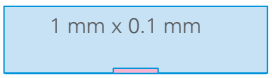
3D Cell culture: interstitial flow

Application: tumor cells in 3D gel matrix (i.e. collagen)
3D cultures of cells and tissue (hepatocytes, fibroblasts, muscle cells, kidney cells, stem cells)
Flow characteristics: continuous interstitial or stop flow interstitial
Recommended pumps: ibidi pump system
Duration: 12 hours up to several weeks
Recommended slides: µ-Slide VI, µ-Slide I Luer
Experimental environment: incubation conditions*

** 37 °C, 5 % CO_2 in air, 90 % rel. humidity*

Example experiments with varying channel geometry

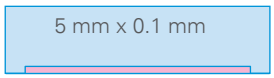
µ-Slide VI 0.1 and µ-Slide III 0.1



0.1 dyne/cm² @ 0.94 µl/min
1 dyne/cm² @ 9.37 µl/min
10 dyne/cm² @ 93.70 µl/min

Exp. volume⁺: 470 µl *
Factor: 1

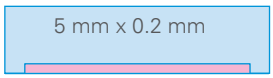
µ-Slide I 0.1 Luer



0.1 dyne/cm² @ 4.94 µl/min
1 dynevcm² @ 49.38 µl/min
10 dyne/cm² @ 493.83 µl/min

Exp. volume⁺: 2469 µl *
Factor: 5.25

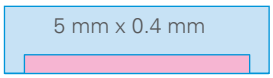
µ-Slide I 0.2 Luer



0.1 dyne/cm² @ 0.02 ml/min
1 dyne/cm² @ 0.19 ml/min
10 dyne/cm² @ 1.95 ml/min

Exp. volume⁺: 9.5ml *
Factor: 20.2

µ-Slide I 0.4 Luer

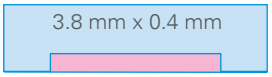


0.1 dyne/cm² @ 0.08 ml/min
1 dyne/cm² @ 0.76 ml/min
10 dyne/cm² @ 7.60 ml/min

Exp. volume⁺: 38 ml
Factor: 80.8

* not recommended for static culture over more than 6 hrs.

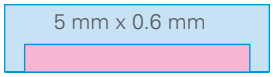
µ-Slide VI 0.4



0.1 dyne/cm² @ 0.06 ml/min
1 dyne/cm² @ 0.57 ml/min
10 dyne/cm² @ 5.68 ml/min

Exp. volume⁺: 28.4 ml
Factor: 60.4

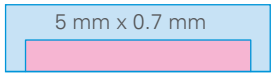
µ-Slide I 0.6 Luer



0.1 dyne/cm² @ 0.17 ml/min
1 dyne/cm² @ 1.66 ml/min
10 dyne/cm² @ 16.64 ml/min

Exp. volume⁺: 83.2 ml
Factor: 177.0

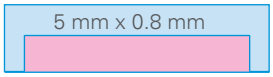
µ-Slide upright 0.7



0.1 dyne/cm² @ 0.22 ml/min
1 dyne/cm² @ 2.23 ml/min
10 dyne/cm² @ 22.34 ml/min

Exp. volume⁺: 111.7 ml
Factor: 237.7

µ-Slide I 0.8 Luer

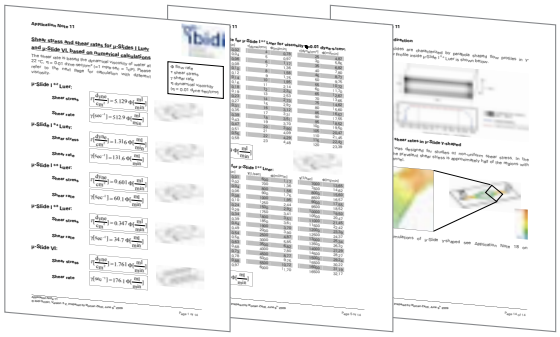


0.1 dyn/cm² @ 0.29 ml/min
1 dyne/cm² @ 2.88 ml/min
10 dyne/cm² @ 28.78 ml/min

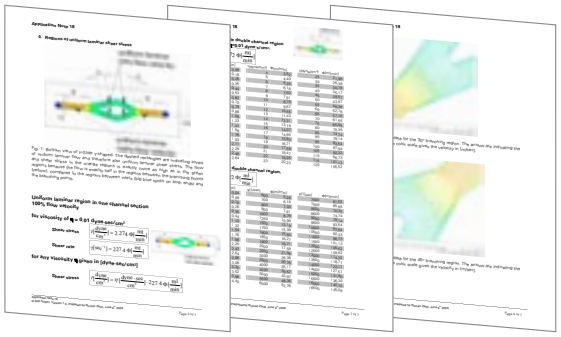
Exp. volume⁺: 143.9 ml
Factor: 306.2

⁺Experimental conditions: Shear stress 10 dyne / cm² | Duration 5 min | Viscosity 0.01 dyne sec / cm²

Application notes for flow assays



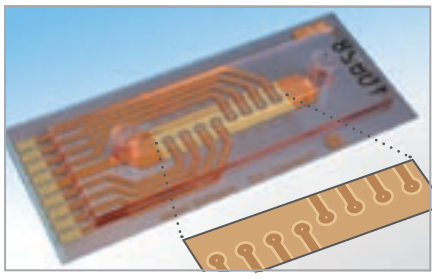
Application Note 11 contains information about shear stress and shear rates in all ibidi channel slides (µ-Slide VI, µ-Slide III, µ-Slide I Luer etc). The data are given in form of formulas and tables. Background information for the calculations is also provided.



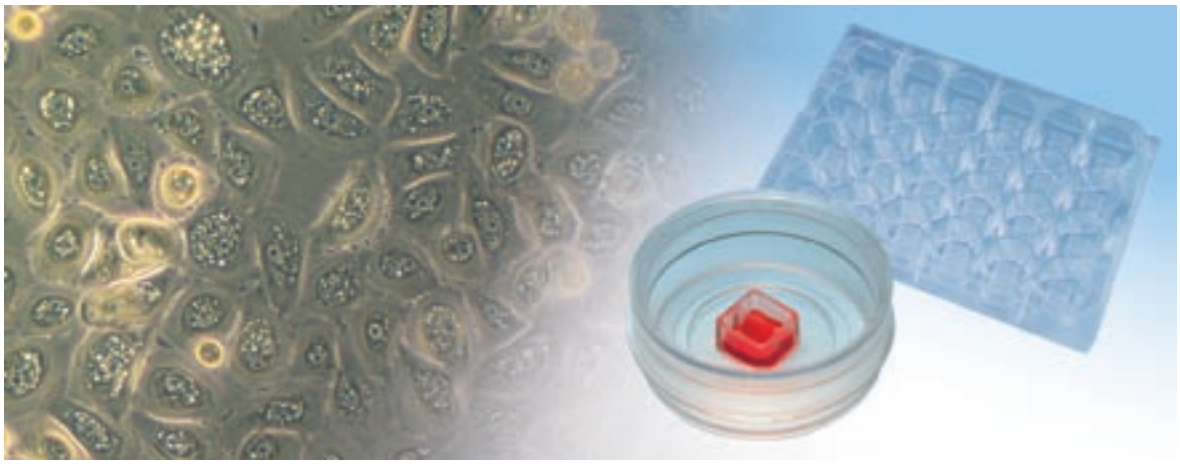
Application Note 18 gives detailed information about the shear stress and shear rates in µ-Slide y-shaped. The data describing the non-uniform flow in the branching regions were calculated by computational fluid dynamics simulation.

ECIS flow module

The ECIS technology allows measuring even small morphological changes with electrical signals, with minimum disturbance, and without any stainings. The measurements are automated and therefore highly reproducible with minimum work.
For more information see pages 104 - 105.

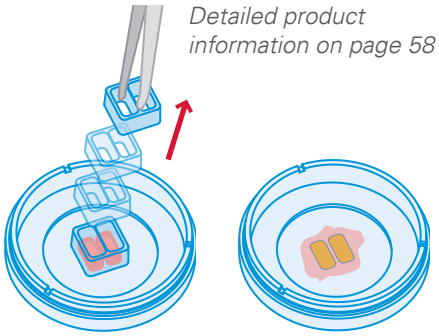


Wound healing & Invasion



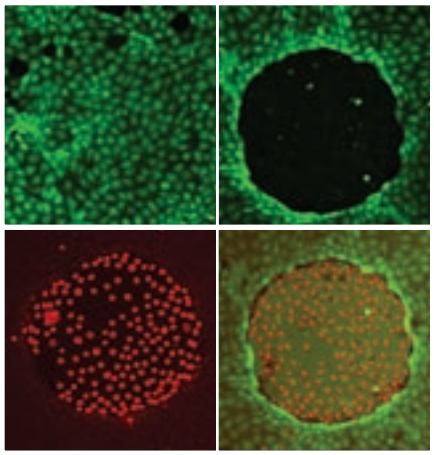
Wound healing and 2D invasion assays are used to screen pharmaceutical substances. The migration behavior under such conditions can be analyzed to investigate cellular behavior on a molecular level. For the mentioned applications microscopy is a key technique. The new ibidi Culture-Insert is compatible with any flat and clean cell culture surface.

- wound healing assays
- invasion assays
- migration assays
- co-cultivation
- defined cell seeding



Detailed product information on page 58

ECIS wounding assay



The ECIS wound healing assay is described on pages 104-105.

Principle and handling

Placed on a cell culture surface, the Culture-Insert provides two cell culture reservoirs. The reservoirs are separated by a 500 µm thick wall. Culturing cells in both reservoirs and removing the silicon insert from the surface results in two well defined cell patches separated by a zone of exactly the same width as the separation wall.

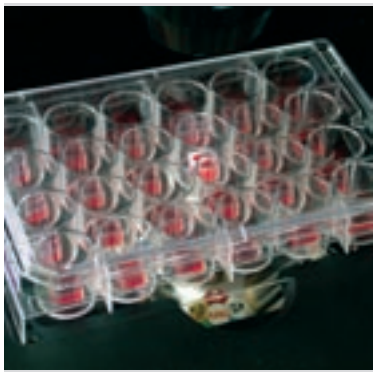
Due to the specially designed bottom, the Culture-Insert will stick to the surface by itself. After removal from the surface the created gap (wound) will be clean and unchanged since no material will remain. The sticky design fully prevents cell growth under the walls. In this manner highly defined regions without cells will be created. The ibiTreat surface provides excellent cell growth in the non-covered areas.

2D invasion assay

The interaction of two different cell types can be investigated by seeding them separately into the two wells. An important example is the invasion of tumor cells in a fibroblast culture. In these assays, cell specific fluorescent labels can be used to distinguish different cell populations.

Comparison of wound healing assays

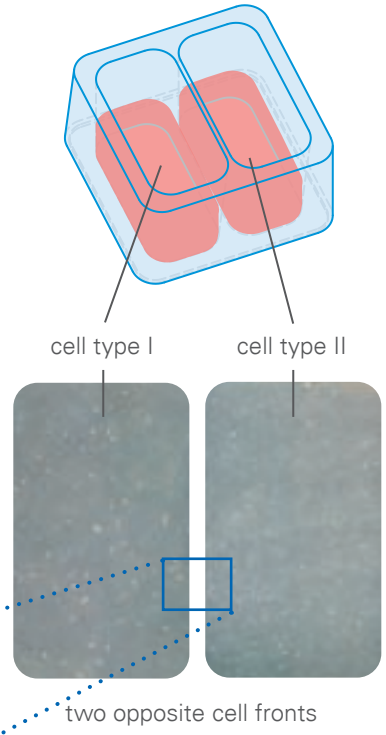
ibidi Culture-Inserts	Scratch assays
Cell seeding into designated areas	Scratching with a needle or tip
Defined cell-free gap	Varying cell-free gap
Defined non-coated surface	Possible extra-cellular matrix remains
No cell damage	Cell damage
Internal reference	No internal reference

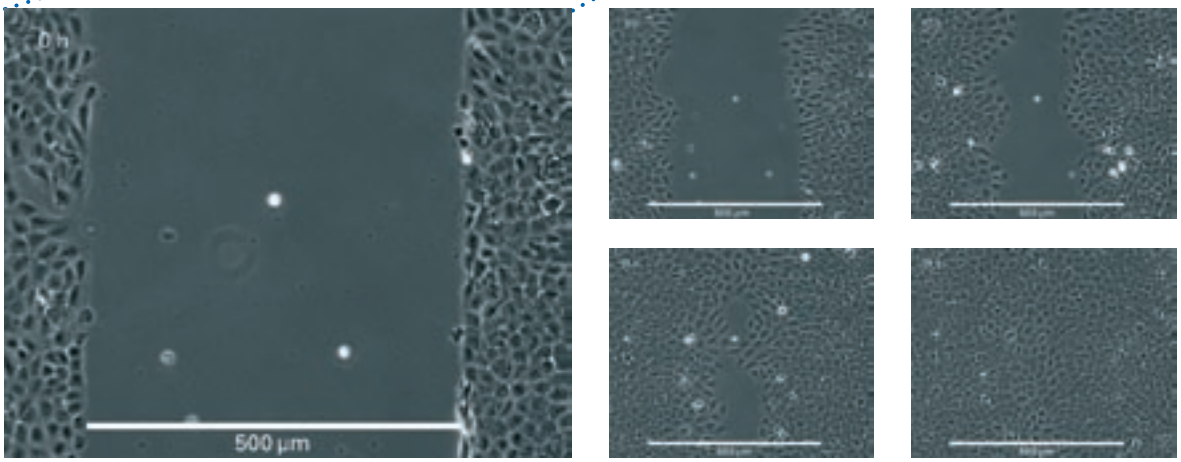


The scratch assay is a widely used technique to investigate wound healing processes. However, it has certain drawbacks in reproducibility. This is mostly due to the fact that scratching might not only remove cells but also coatings from the surface. Even microscopic defects of several µm in depth might appear. The width of the scratch generally depends on user specific parameters.

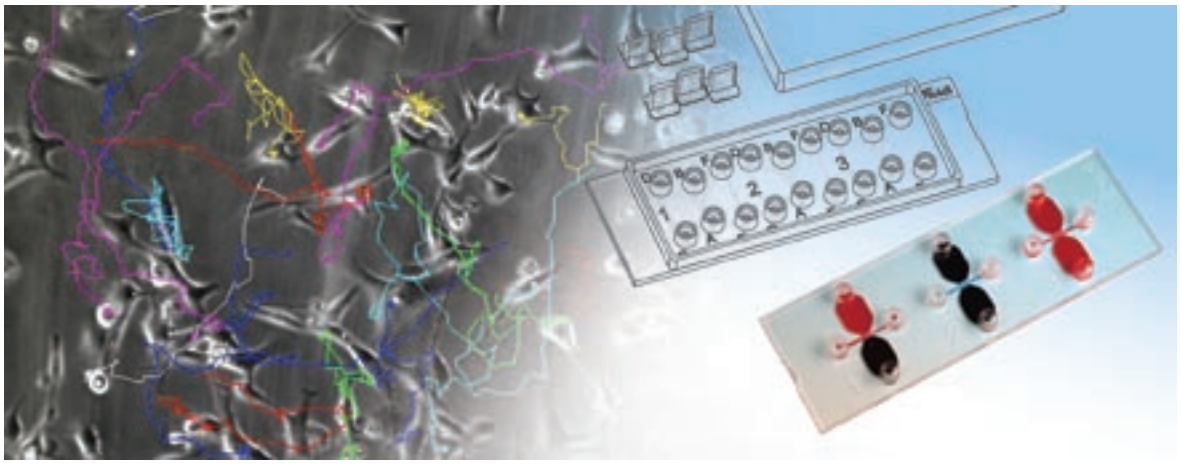
The Culture-Insert, in combination with the ibiTreat surface of the µ-Dish, overcomes the problems of the scratch assay. When the insert is removed there is no change to the ibiTreat surface. This has been proven in tests with different kinds of cells like fibroblasts, keratinocytes, and several endothelial cell types.

In case of protein adhesion coatings, we recommend performing suitable reference measurements in order to exclude effects on wound healing results generated from removing protein matrix together with the Culture-Insert.





Chemotaxis



Concentration gradients of a large variety of substances induce a directed motion of cells (chemo-taxis). Due to its importance for angiogenesis, oncology, neurology, and especially immunology, the question of migration under a special stimulation gains a lot of interest. Until now, there was no simple system to study these complicated correlations in easy optical assays.

ibidi chemotaxis slides

To overcome the disadvantages of existing chemotaxis assays, the ibidi research team developed µ-Slides for chemotactical analysis. The special chambers allow high resolution microscopy, convenient liquid handling, and live cell imaging under defined linear concentration gradients.

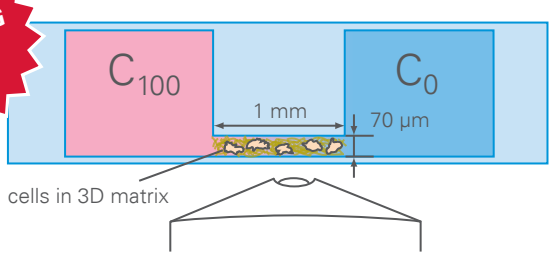
The ibidi chemotaxis system provides answers to the following experimental questions:

- chemotactic activity
- strength of chemotactic activity
- influence of substances on chemotactical activity
- cell morphology and viability
- cell-cell interaction

µ-Slide Chemotaxis 3D

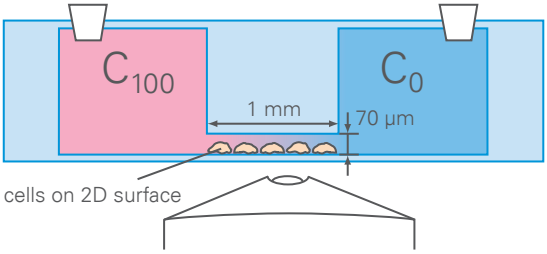
The µ-Slide Chemotaxis 3D was developed for investigating the chemotactical behavior of non-adherent cells in gel matrices. It is possible to observe the migration in linear and stable concentration profiles over more than 48 hours. Also fast responses within less than 30 min can be measured as gradients can be rapidly established.

COMING SOON



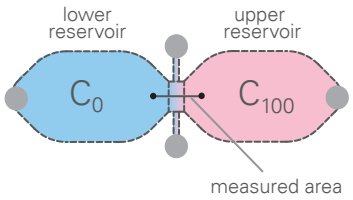
µ-Slide Chemotaxis

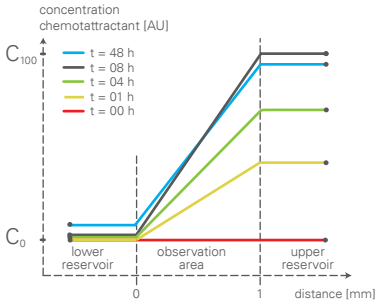
The µ-Slide Chemotaxis is optimized for analyzing the chemotactical response of adherent cells in linear and stable concentration profiles. Due to the gradient's time stability of over 48 hours it is the first system able to analyze chemotaxis of slow migrating cells over days.



Gradient stability

The gradient formed in µ-Slide Chemotaxis provides a linear profile over the observation area. It is stable for at least 48 hours. Such a time period also allows slowly migrating cells to migrate significant distances.

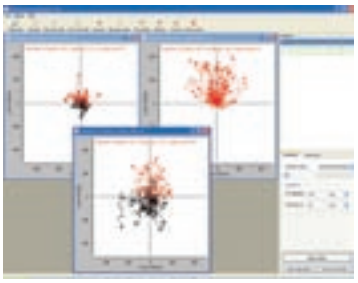




Data analysis – "Chemotaxis and Migration Tool"

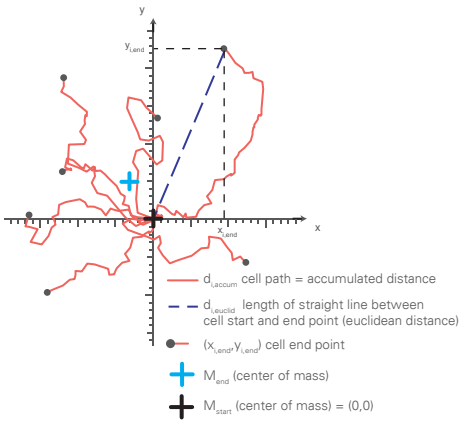
For data analysis from chemotaxis experiments (time stacks) ibidi has developed a free software analysis tool. This 'Chemotaxis and Migration Tool' now runs as an independed program which is freely available on www.ibidi.com. The tool provides different types of graphs and statistical tests to perform advanced analysis of experimental data.

After cell tracking, the cells' paths can be plotted and analyzed for chemotactical effects. An easy microscopic calibration gives access to a variety of parameters. For specific analyses there are sector tools, able to count cells in angular or circular areas, so called Rose plots. A statistical test for inhomogeneity of cell distribution (Rayleigh test) completes the software.



All calculations can be directly visualized by histograms and diagrams. Optionally, all data can be exported for further analysis. With a complete set of migrational data, the user is able to quantify chemotaxis and random migration.

The software and a pdf documentation are freely available on www.ibidi.com



Chemotaxis parameters

For quantification of chemotaxis and migration several values can be generated by the software tool. The center of mass and the y and x Forward Migration Indices are a measure of directed cell migration. The directionality on the other hand must not necessarily indicate a chemotaxis effect.

Center of mass (M_{end})

The center of mass represents the averaged point of all cell endpoints. Its x and y values indicate in which direction the group of cells mainly drifted.

$$M_{end} = \frac{1}{n}\sum_{i=1}^{n}(x_{i,end}, y_{i,end})$$

i is the index of the different single cells. The first cell has the index 1, the last one n.

$1 \le i \le n$ *index of the cells*

x and y Forward Migration Indices (x_{FMI}, y_{FMI})

The x_{FMI} and y_{FMI} represent the efficiency of forward migration of cells relating to the x or y axis. The larger the index on an axis the stronger the chemotactic effect on this axis. For simplification it is assumed that either the x-axis or the y-axis are parallel to the direction of the chemotactic gradient.

$$x_{FMI} = \frac{1}{n}\sum_{i=1}^{n}\frac{x_{i,end}}{d_{i,accum}}$$

$$y_{FMI} = \frac{1}{n}\sum_{i=1}^{n}\frac{y_{i,end}}{d_{i,accum}}$$

Directionality (D)

The directionality is calculated by comparing euclidian and accumulated distance. It is a measure of directness of cell trajectories. A directionality of D -> 1 means a straight-line migration from start to endpoint.

directionality of one single cell

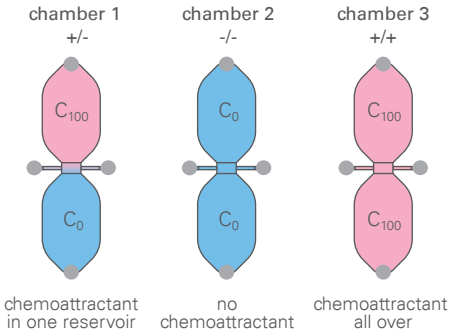
$$D_i = \frac{d_{i,eucld}}{d_{i,accum}}$$

averaged directionality of all cells

$$D = \frac{1}{n}\sum_{i=1}^{n} D_i = \frac{1}{n}\sum_{i=1}^{n}\frac{d_{i,eucld}}{d_{i,accum}}$$

Chemotaxis – example experiment

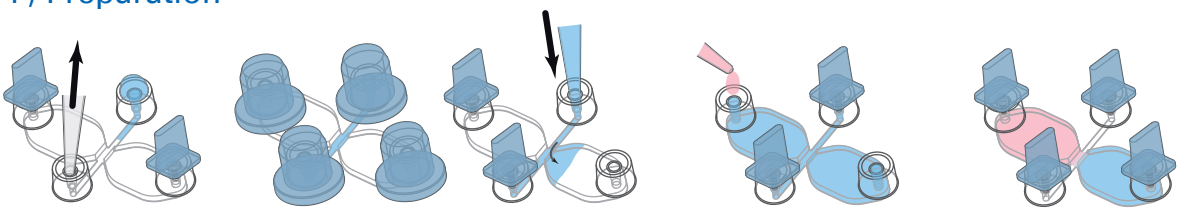
The following example experiment was performed with the chemotactic cell line HT1080 (human fibrosarcoma) with serum as a chemoattractant.



0) Experimental parameters

Cells: HT1080 / seeding density: 3 x 10^6 cells/ml
Slide: µ-Slide Chemotaxis, Collagen IV
Seeding medium: DMEM (10% FCS)
Starvation medium: DMEM (without FCS) [-]
Attractant medium: DMEM (10% FCS) [+]
Adhesion time: 2 h
Chamber 1: reservoir 1 + / reservoir 2 -
Chamber 2: reservoir 1 - / reservoir 2 -
Chamber 3: reservoir 1 + / reservoir 2 +
Experimental time: 24 h

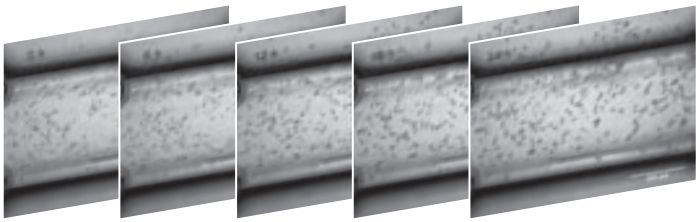
1) Preparation



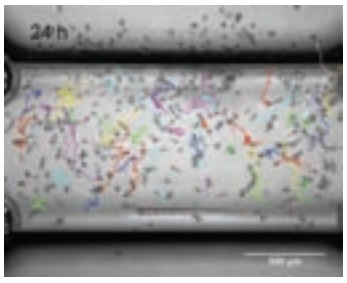
seed cells | incubation | fill the chamber with DMEM without FCS | fill 18 µl DMEM with 10% FCS into the chamber | ready for video microscopy

2) Video microscopy

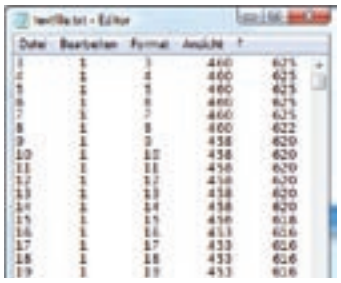
Video microscopy is an absolutely necessary tool for ibidi's chemotaxis and migration assays. Without video microscopy there is no access and analysis of chemotaxis effects.



3) Cell tracking



Cells' traces are visualized after tracking with ImageJ plugin "Manual Tracking".



Data table with (x, y) positions for each point of time (t).

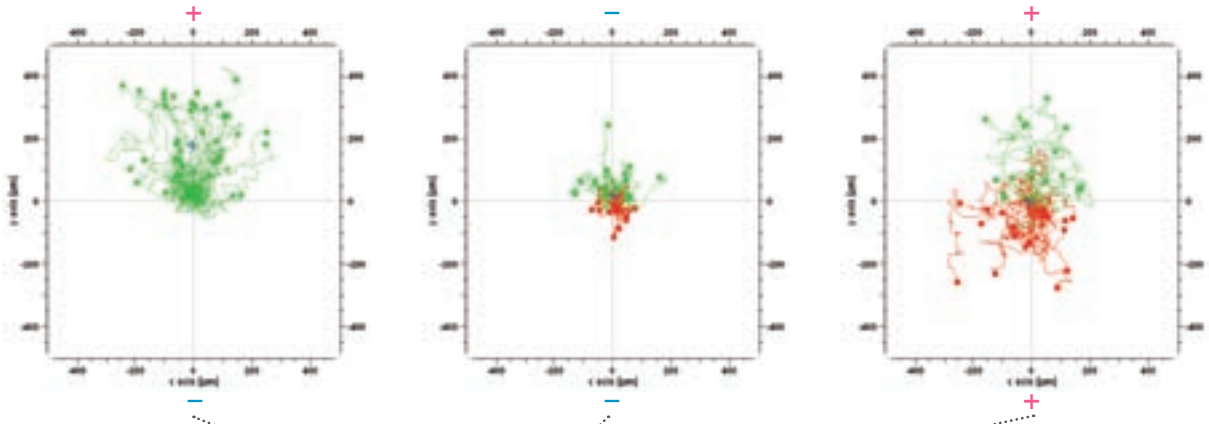
Only tracking cells provides access to quantification of cell movement between frames of a temporal stack. Usually, tracking is done manually or automatically by special tracking software. Automated tracking algorithms need distinct objects, i.e. fluorescent labeled cells. After tracking the cells´ traces their (x, y) values are available for each point of time (t).

Support

Please visit **www.ibidi.com** for application notes, coating protocols, example data, and handling videos of the chemotaxis assay.

4) Plotting the data

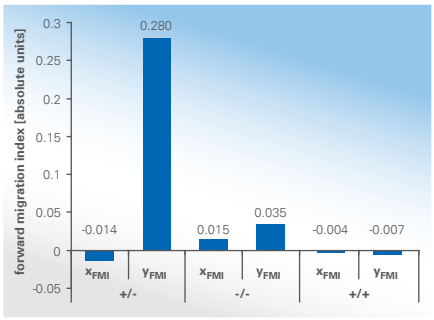
After the coordinate transformation all initial points are set to (0, 0) automatically by the ibidi Chemotaxis and Migration Tool. Cell trajectory plots can easily be created. For better visualization of chemotaxis effects additional information can be added to the plots, e.g. color information of cells moving up/down.



5) Chemotaxis values

Center of mass x [µm]	-4.0	3.6	-7.6
Center of mass y [µm]	176.3	18.1	-3.1
Center of mass lenght [µm]	176.4	18.5	8.2
x_{FMI}	-0.014	0.015	-0.004
y_{FMI}	0.280	0.035	-0.007
Directionality D	0.33	0.16	0.21
Mean eucledian distance [µm]	208.9	65.8	145.8
Mean accumulated distance [µm]	617.1	411.8	716.4
Cell velocity [µm/min]	0.43	0.29	0.49
Rayleigh test	< 0.05	>0.05	>0.05

All desired chemotaxis values can easily be generated by the Chemotaxis and Migration Tool.



6) Interpretation of data

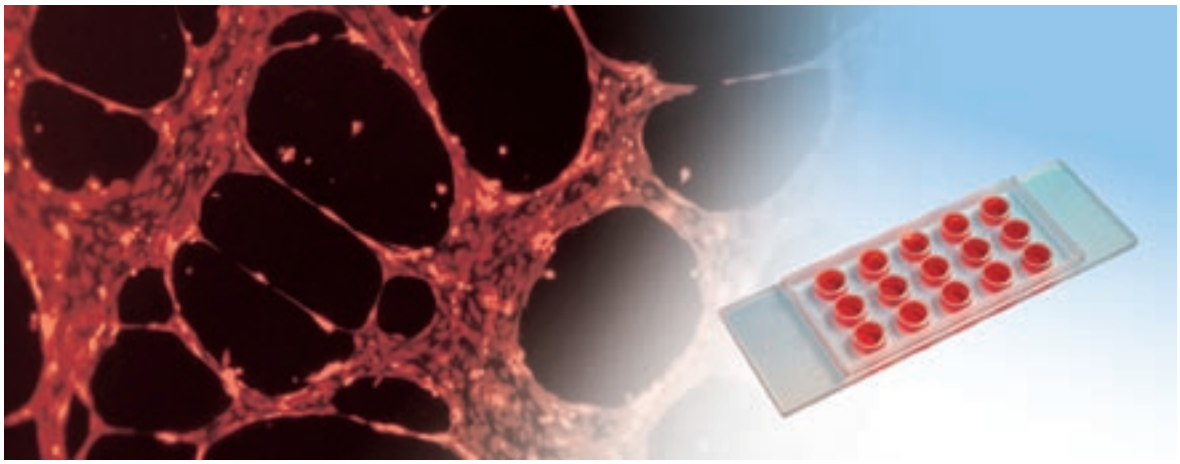
Simple interpretation

The easiest way of data interpretation is a visual inspection of the cell trajectories (plots). Strong and significant chemotaxis effects towards one specific direction can be easily seen. Furthermore, significant differences in cell velocity and directionality between chemotaxis and control experiment can be distinguished. When dealing with strong chemotaxis effects, like in our example, or total chemotaxis inhibition, this simple interpretation might be sufficient. We also recommend taking the provided Rayleigh test into account. In our example you can also see that the total amount of chemoattractant, without any gradient, does lead to a different migration behavior of the cells.

Advanced interpretation

In addition to the optical impression of the plots, profound parameters can be used to prove a chemotaxis effect or a hypothesis. Parameters like the displacement of the center of mass (M) or the forward migration indices are valid measures when they are compared to the right reference measurements. In our example experiment the y_{FMI} of the (+/-) measurement is significantly higher than the x_{FMI} and also than the x_{FMI} and y_{FMI} of reference measurements (-/-) and (+/+).

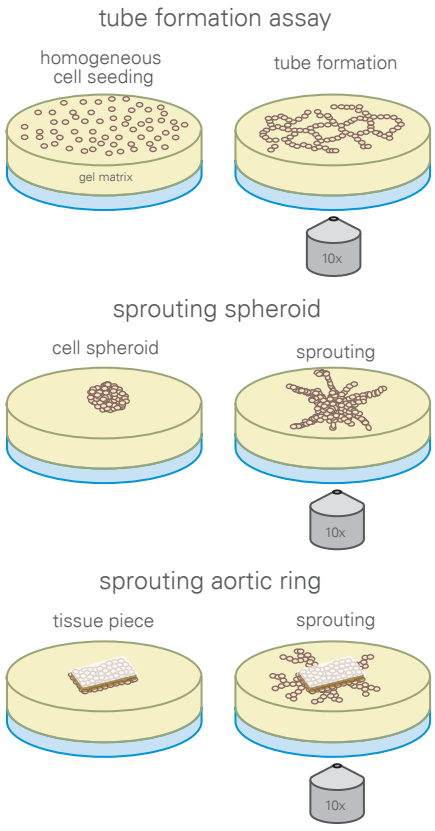
Angiogenesis



If individual tumor cells grow into a macroscopic tumor they drastically increase their demand for oxygen and nutrients. An important example of angiogenesis is the generation of new blood vessels to feed a tumor. *In vitro* angiogenesis assays provide a better understanding of these processes on a cellular and molecular level.

The µ-Slide Angiogenesis was designed for biomedical and pharmaceutical research. 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.

in vitro Angiogenesis assays



Detailed product information on page 63

Angiogenesis assays *in vitro*

Cells on gel matrices can be used to monitor their ability to form new vessels. The tube formation assay is done by seeding single cells and observing characteristic patterns. To carry out the sprouting assays either spheroids or pieces of tissue e.g. from aorta are placed on the gel matrix. All these assays share a need for a well defined thickness of the gel layer underneath.

The assays performed in the µ-Slide Angiogenesis benefit from a well defined thickness of the gel matrices. Besides reproducible cell culture conditions, the cells are placed in one optical plane.

Optical improvements

The µ-Slide Angiogenesis was primarily developed to simplify the tube formation assay of endothelial cells on Matrigel®. This kind of assay is usually done in multi-well plates. In large wells, such as 6 or 12 well plates, the great amount of gel matrix required is very expensive. Using smaller wells causes a meniscus formation of the gel surface which leads to inhomogeneous cell distribution patterns. Also, the meniscus does not allow the cells to be in one optical plane.

Matrigel® is a registered trade mark of Becton, Dickinson and Company

Another meniscus (formed by the air-liquid interface) restricts phase contrast to a small area in the center of the well.

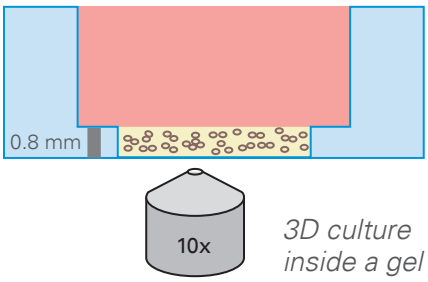
The µ-Slide Angiogenesis solves these problems providing a 4 mm well in a 5 mm well. This optical improvement is called "well in a well".

Filling the smaller well with exactly 10 µl of gel will result in a plane gel surface. Filling the major well with exactly 50 µl also avoids meniscus formation on the air-liquid interface.

3D Cell cultures

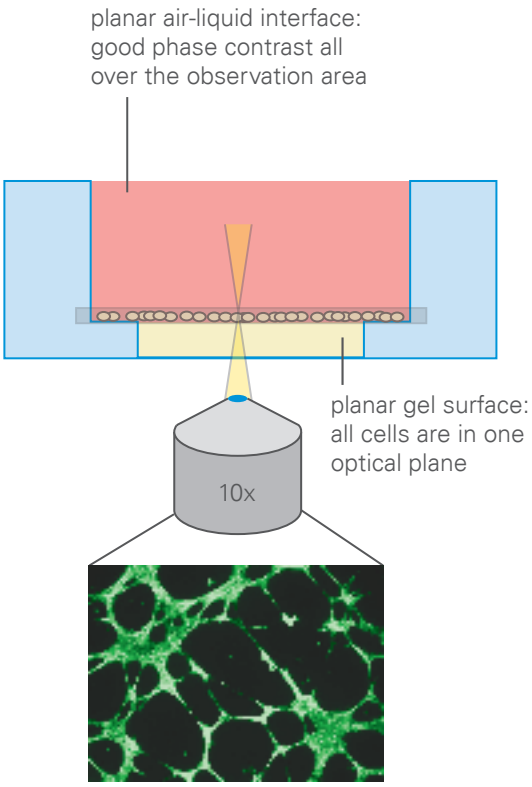
The "well in a well" feature of the µ-Slide Angiogenesis also supports the microscopy of cells embedded in gel matrices. 3D cell cultures mimick *in vivo* conditions e.g. of cancer cells and hepatocytes. Also non-adherent cells such as blood cells or bacteria can be immobilized for enhanced microscopy access.

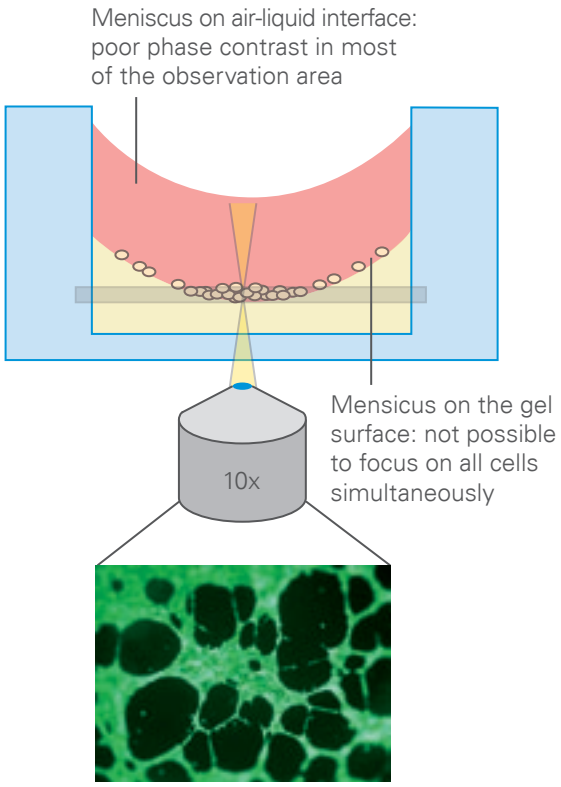
The amounts of gel matrix and medium volume are balanced for the nutrient supply of those 3D cell cultures with low and medium cell densities.



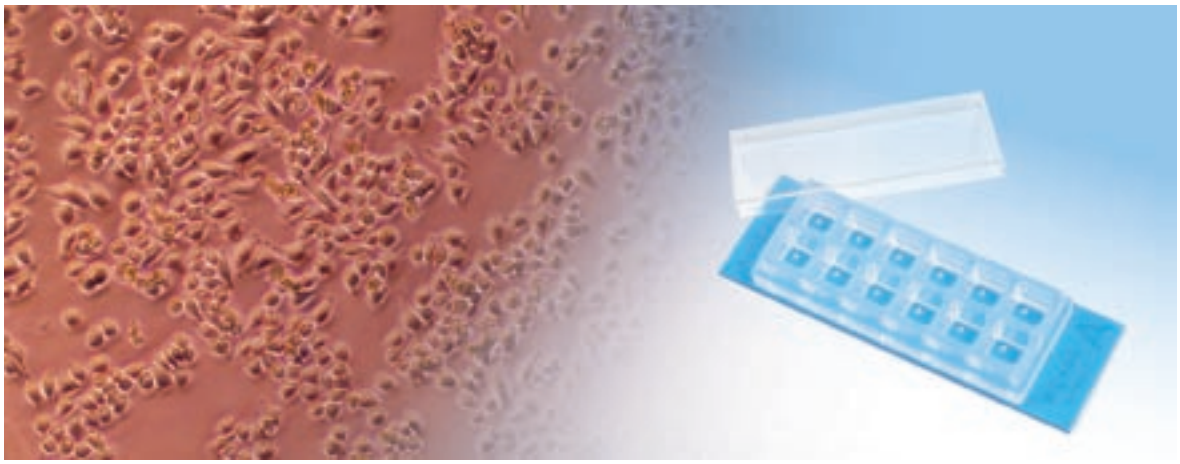
3D culture inside a gel

µ-Slide Angiogenesis vs. standard well



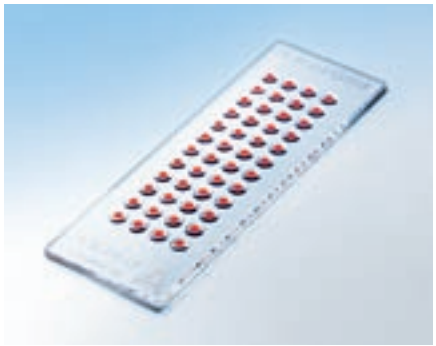


Cell Culture-to-PCR



Conventional cell culture, treatment and extraction for gene expression analysis may induce cellular stress responses which affect gene expression. In addition, current methods require high volume culture flasks and spin-column extractions which are time-consuming and costly. The innovative AmpliCell system, which contains the AmpliCell slide and AmpliSpeed slide cycler, integrates culturing, treatment and PCR on a single platform without extraction. Minimize cellular stress and save time and reagents for transfection, microRNA assays, and methylation analysis.

Cell culture in AmpliCell chamber: AmpliGrid PCR slide with 12-chamber upper structure



48 PCR reactions on AmpliCell (after removal of upper structure and excess media)

Cell cultivation and PCR on AmpliCell

The AmpliCell slide consists of AmpliGrid, a glass slide for 1 µl PCR reactions, plus a removable upper structure that sits tightly sealed on top of the slide and separates 12 growth chambers. Choose from 3 surface coatings (fibronectin, collagen, poly-L-lysine) for optimal growth conditions. After cell expansion, remove the upper chamber structure, wash off excess media, and run up to 4 PCR reactions per chamber.

Details at: www.advalytix.com/amplicell

Transfection-to-PCR

Conventional transfection assays require large reagent volumes to ensure optimal growth conditions and extraction adds to process time and cost. AmpliCell provides optimal growth conditions with only 100 µl reagent volume which saves up to 90 % reagent costs. No extraction is needed further reducing process time and costs. Workflows: (1) grow cells first then apply transfection reagent, (2) reverse approach: apply transfection reagent without prior cell culture to allow up to 3 runs per week. Take advantage of AmpliCell's optical control of cell morphology and transfection efficiency prior to PCR: What you see is what you amplify!

AmpliCell is an open platform and for use with commercially available transfection chemistries. Transfection application report at:

www.advalytix.com/images/downloads/AR_AmpliCell.pdf

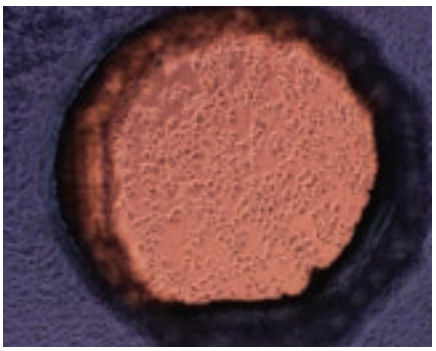
Methylation on the AmpliCell

Conventional methylation methods require multiple sample transfers which induces cell stress that can bias methylation results; they are also time consuming and costly. With AmpliCell, carry out complete cell growth, treatment and sequencing reaction workflow on the same substrate: (1) cultivate cells on AmpliCell and apply bisulfite treatment in a 1µl reaction, (2) visually control cells, (3) amplify gene(s) of interest and run sequencing reaction (no extraction needed), (4) remove samples to analyze results.

Methylation application report:

www.advalytix.com/images/downloads/AR_Methylation.pdf

Optical properties



Ever tried to detect cells in the opaque 3D structure of a microplate? AmpliCell greatly simplifies cell detection because cells are neatly arranged in a monolayer on top of a high quality and ultra-low fluorescence glass substrate. The high optical quality of the AmpliCell slide allows superior control of cell seeding, cell growth and assay success prior to PCR by standard and fluorescent microscopy or image acquisition using fluorescent scanners.

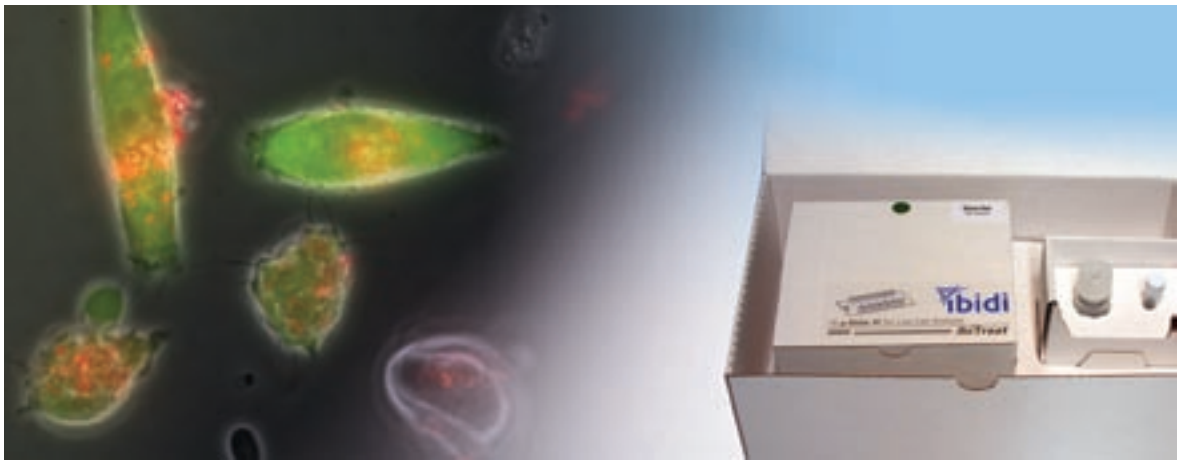
What you see is what you amplify!

Sensitive PCR without sample preparation

After cell expansion, remove the upper chamber structure, wash off excess cells, and run up to 4 independent (RT)-PCR reactions and optional subsequent qPCR on the cells in each chamber. Amplification takes place in 1 µl master mix volumes on each reaction site. Reactions are covered with 5µl of sealing solution (delivered with the AmpliCell) to prevent evaporation during thermal cycling. The perfect solution to thermo cycle or incubate AmpliCell slides is the AmpliSpeed slide cycler.

Details at *www.advalytix.com/amplispeed*

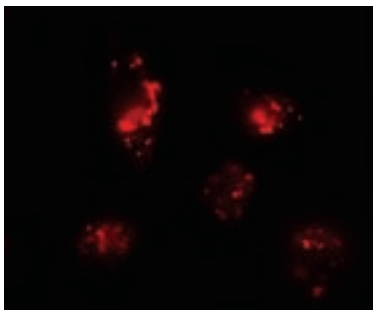
Transfection and proteofection

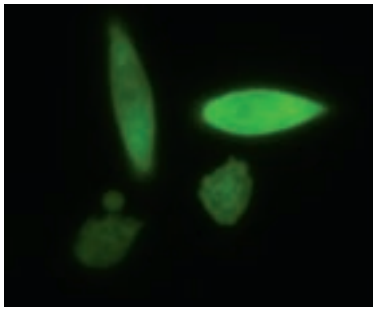


Transfection

Transfection is the process of inserting "foreign" genetic material, such as plasmid DNA or si-RNA into cells. The process typically involves opening transient pores or "holes" in the cell membrane, to allow the uptake of such material. As the pure genetic material would not be transported to such pores, it is normally covered with a transfection agent, classically calcium phosphate, dextrans, cationic polymers or lipids.

The ratio of transfection material and the transfection agent is critical – since the procedures interfere with the cell motility and ratio per area might play a more crucial role – than pure concentration. We therefore developed a protocol and an ideal reagent – a cationic lipid – to perform transfection in ibidi µ-Slides.





Advantages

- cell culture, transfection, and microscopy – all-in-one slide
- METAFECTENE® µ designed specifically for the µ-Slide VI 0.4
- fast and simple protocol – no optimization required
- fast results – two experiments per week
- 90 transfection experiments per kit
- non viral transfection

Advantages fluorescence version

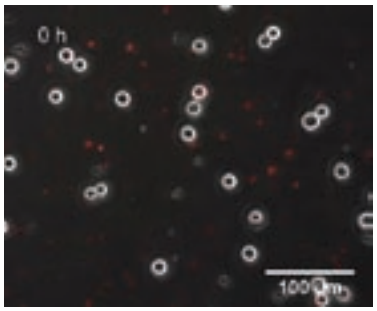
- METAFECTENE® µ FluoR is a Rhodamine labeled transfection reagent
- same protocol as with METAFECTENE® µ
- allows to follow the uptake and the fate of the transfection reagent

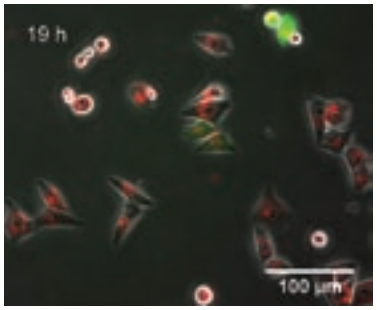
Applications

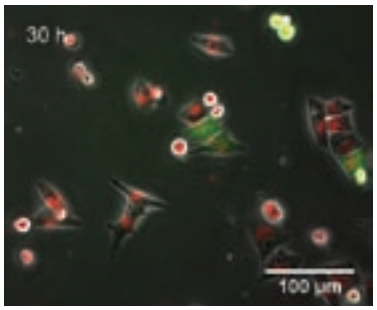
- imaging of transfected fluorescence proteins
- protein localization with fluorescence labels
- RNA-silencing by miRNA and siRNA
- transient and stable gene transfection

Proteofection

Proteofection is built on the transfection idea but instead of the insertion of plasmid DNA, proteins/antibodies are directly inserted into the cells and transcription is not required. After endocytosis the proteins are structurally and functionally unchanged and can act directly within the cells. These innovative new reagents open up new research possibilities in the growing field of proteomics. For example, intra-cellular transport of the protein responsible for apoptosis can help to understand programmed cell death mechanisms and drug resistance.







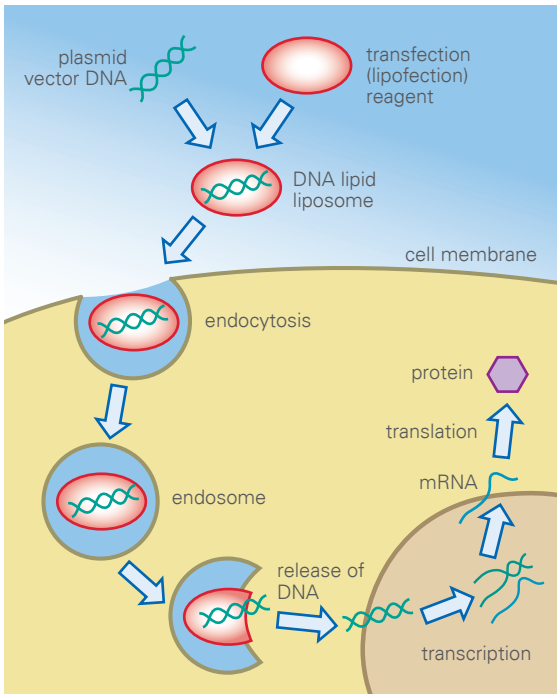
Advantages

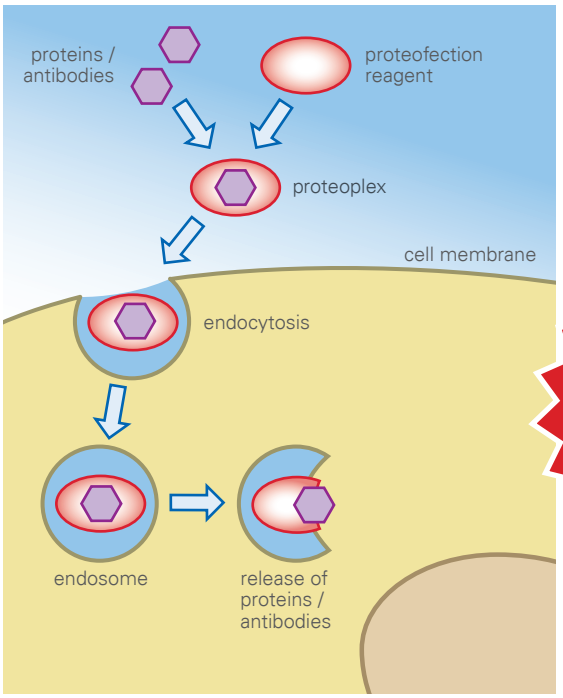
- cell culture, protein uptake, and microscopy – all-in-one slide
- kit contains µ-Slide VI $^{0.4}$ and PROTEOfectene®
- fast and simple protocol
- quickly generated results via direct protein uptake
- 90 experiments per kit

Applications

- protein localization through fluorescent antibodies
- intracellular competetive inhibition
- replacing proteins and protein studies in knock down organisms

Transfection vs. Proteofection





Deliver proteins and antibodies directly into living cells

Selected publications with ibidi products

The following selected scientific publications cite the use of ibidi products:

Live cell imaging / video microscopy

Renaud, J., G. Kerjan, I. Sumita, Y. Zagar, V. Georget, D. Kim, C. Fouquet, K. Suda, M. Sanbo, F. Suto, S.L. Ackerman, K.J. Mitchell, H. Fujisawa, and A. Chedotal; Plexin-A2 and its ligand, Sema6A, control nucleus-centrosome coupling in migrating granule cells. Nature Neuroscience, 2008

Pohl, C., and S. Jentsch; Midbody ring disposal by autophagy is a post-abscission event of cytokinesis. Nature Cell Biology, 2008

Winderlich, M., L. Keller, G. Cagna, A. Broermann, O. Kamenyeva, F. Kiefer, U. Deutsch, A.F. Nottebaum, and D. Vestweber; VE-PTP controls blood vessel development by balancing Tie-2 activity. Journal of Cell Biology, 2009

Hübner, W., G.P. McNerney, P. Chen, B.M. Dale, R.E. Gordon, F. Chuang, X.D. Li, D.M. Asmuth, T. Huser, and B.K. Chen; Quantitative 3D video microscopy of HIV transfer across T cell virological synapses. Science, 2009.

Shear stress and cells under flow

Kotsis, F., R. Nitschke, M. Doerken, G. Walz, and E.W. Kuehn; Flow modulates centriole movements in tubular epithelial cells. Pflügers Archiv European Journal of Physiology, 2008

Pfaff, D., M. Heroult, M. Riedel, Y. Reiss, R. Kirmse, T. Ludwig, T. Korff, M. Hecker, and H.G. Augustin; Involvement of endothelial ephrin-B2 in adhesion and transmigration of EphB-receptor-expressing monocytes. Journal of Cell Science, 2008

Cicha I., Beronov K., Ramirez E. L., Osterode K., Goppelt-Struebe M., Raaz D., Yilmaz A., Daniel W. G. and Garlichs C. D.; Shear stress preconditioning modulates endothelial susceptibility to circulating TNF-[alpha] and monocytic cell recruitment in a simplified model of arterial bifurcations. Atherosclerosis, 2009

Immunofluorescence

Masamune, A., K. Kikuta, T. Watanabe, K. Satoh, A. Satoh, and T. Shimosegawa; Pancreatic stellate cells express Toll-like receptors. Journal of Gastroenterology, 2008

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Cell culture in 2D and 3D

Tournaviti, S., S. Hannemann, S. Terjung, T.M. Kitzing, C. Stegmayer, J. Ritzerfeld, P. Walther, R. Grosse, W. Nickel, and O.T. Fackler; SH4-domain -induced plasma membrane dynamization promotes bleb-associated cell motility. Journal of Cell Science, 2007

Shields J.D., Fleury M.E., Yong C., Tomei A.A., Randolph G.J., and Swartz M.A.; Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling. Cancer Cell, 2007

Co-Culture

Singh, S.K., C. Nizard, R. Kurfurst, F. Bonte, S. Schnebert, and D.J. Tobin; The silver locus product (Silv/gp100/Pmel17) as a new tool for the analysis of melanosome transfer in human melanocyte-keratinocyte co-culture. Experimental Dermatology, 2008

Scheubel, R.J., J. Holtz, I. Friedrich, J. Borgermann, S. Kahrstedt, A. Navarrete-Santos, R.E. Silber, and A. Simm; Paracrine effects of CD34 progenitor cells on angiogenic endothelial sprouting. International Journal of Cardiology, 2008

Transfection

Heuer, D., A.R. Lipinski, N. Machuy, A. Karlas, A. Wehrens, F. Siedler, V. Brinkmann, and T.F. Meyer; Chlamydia causes fragmentation of the Golgi compartment to ensure reproduction. Nature, 2008

Fu, Q., C. Wu, Y. Shen, S. Zheng, and R. Chen; Effect of LIMK2 RNAi on reorganization of the actin cytoskeleton in osteoblasts induced by fluid shear stress. Journal of Biomechanics, 2008

Neuronal cell research

Santos S.D.M., Verveer P.J., and Bastiaens P.I.H.; Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate. Nature Cell Biology, 2007

Nakano-Kobayashi A., Yamazaki M., Unoki T., Hongu T., Murata C., Taguchi R., Katada T., Frohman M.A., Yokozeki T., and Kanaho Y; Role of activation of PIP5Kã661 by AP-2 complex in synaptic vesicle endocytosis. The EMBO Journal, 2007

Stem cell research

Morosetti, R., M. Mirabella, C. Gliubizzi, A. Broccolini, C. Sancricca, M. Pescatori, T. Gidaro, G. Tasca, R. Frusciante, and P.A. Tonali; Isolation and characterization of mesoangioblasts from Facioscapulohumeral Muscular Dystrophy Muscle Biopsies. Stem Cells, 2007

Lippuner, C., D. Paape, A. Paterou, J. Brand, M. Richardson, A.J. Smith, K. Hoffmann, V. Brinkmann, C. Blackburn, and T. Aebischer; Real-time imaging of Leishmania mexicana-infected early phagosomes: a study using primary macrophages generated from green fluorescent protein-Rab5 transgenic mice. FASEB, 2008

Chemotaxis

Eiseler, T., H. Doppler, I.K. Yan, K. Kitatani, K. Mizuno, and P. Storz; Protein kinase D1 regulates cofilin-mediated F-actin reorganization and cell motility through slingshot. Nature Cell Biology, 2009

Molina-Ortiz, I., R.A. Bartolome, P. Hernandez-Varas, G.P. Colo, and J. Teixido; Overexpression of E-cadherin on melanoma cells inhibits chemokine-promoted invasion involving p190RhoGAP/p120ctn-dependent inactivation of RhoA. Journal of Biological Chemistry, 2009

Cell tracking and data analysis

Lämmermann, T., B.L. Bader, S.J. Monkley, T. Worbs, R. Wedlich-Soldner, K. Hirsch, M. Keller, R. Forster, D.R. Critchley, R. Fässler, and M. Sixt; Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature, 2008

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Angiogenesis

Rothmeier, A.S., I. Ischenko, J. Joore, D. Garczarczyk, R. Furst, C.J. Bruns, A.M. Vollmar, and S. Zahler; Investigation of the marine compound spongistatin 1 links the inhibition of PKC{alpha} translocation to nonmitotic effects of tubulin antagonism in angiogenesis. FASEB, 2008

Petoumenos, V., G. Nickenig, and W. Nikos; High density lipoprotein exerts vasculoprotection via endothelial progenitor cells. Journal of Cellular and Molecular Medicine, 2008

Wound healing

Park, S.-A., H.-K. Na, E.-H. Kim, Y.-N. Cha, and Y.-J. Surh; 4-Hydroxyestradiol induces anchorage-independent growth of human mammary epithelial cells via activation of I{kappa}B kinase: Potential role of reactive oxygen species. Cancer Research, 2009

Horst, D., S.K. Scheel, S. Liebmann, J. Neumann, S. Maatz, T. Kirchner, and A. Jung; The cancer stem cell marker CD133 has high prognostic impact but unknown functional relevance for the metastasis of human colon cancer. The Journal of Pathology, 2009

Electron microscopy

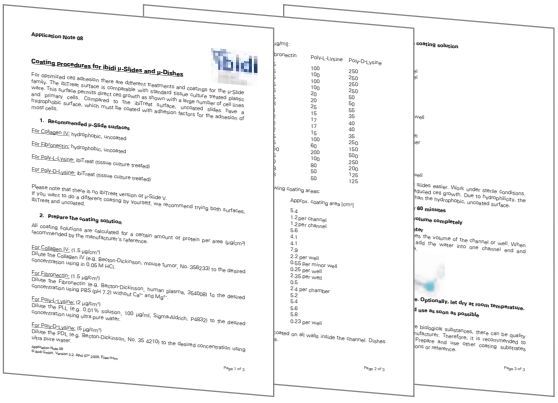
Chappell, D., M. Jacob, O. Paul, M. Rehm, U. Welsch, M. Stoeckelhuber, P. Conzen, and B.F. Becker; The glycocalyx of the human umbilical vein endothelial cell. An impressive structure ex vivo but not in culture. Circulation Research, 2009

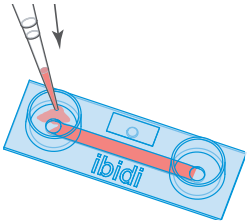
For more publications with ibidi µ-Slides and a keyword database please visit **www.ibidi.com**

Application notes and movies

In our own studies and/or in cooperation with partners, we developed numerous application notes. Together with approximately 300 scientific papers these are powerful sources for your research.

Find a small selection on supporting materials here – and much more on the ibidi website. Today also scientific movies either showing results or handling are a great option for better support. We are presently working on a movie gallery. Please send us your video!

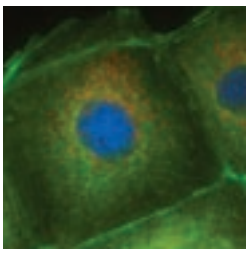




Cell culture coating

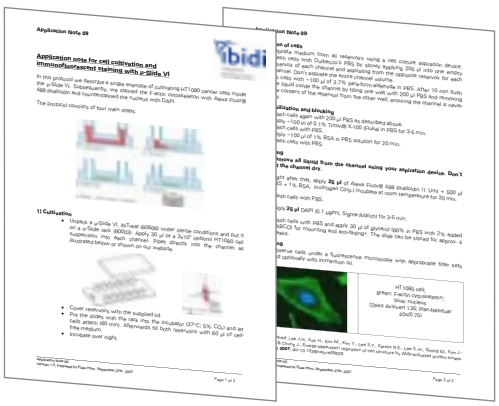
Here you will find exact information on how to make a coating on µ-Slides yourself.

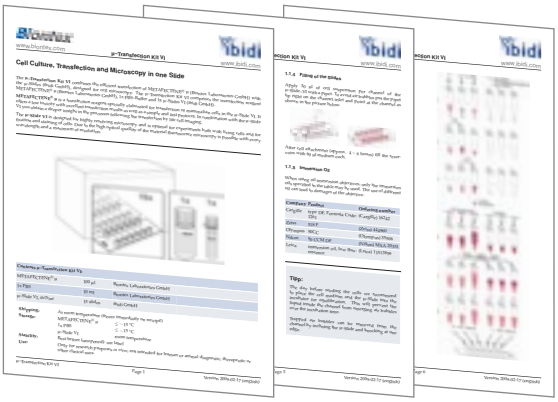
Find many more on the website

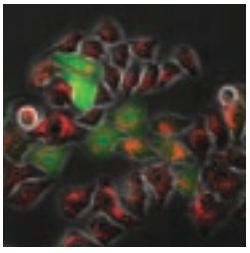


Fluorescence staining

In these examples we describe how to do an immunofluorescence staining using various µ-Slides.

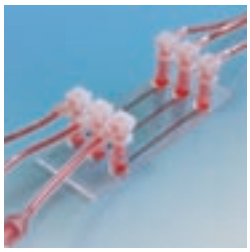






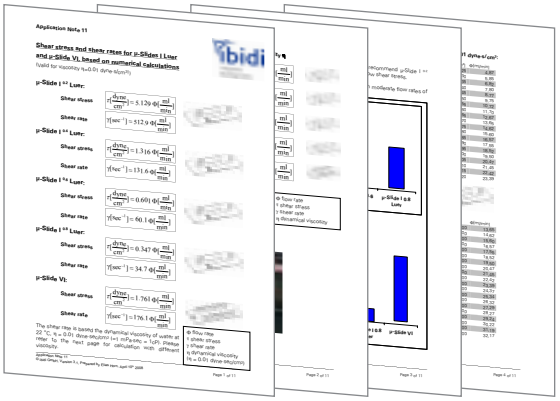
Transfection

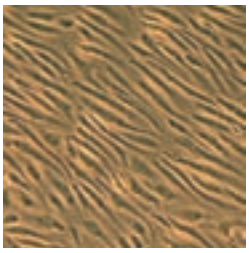
This example shows how transfections can be adapted to work in cell culture channels.



Shear stress and shear rates

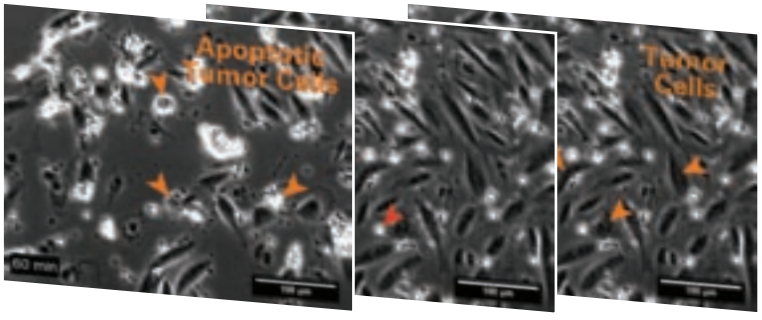
Here you will find detailed information on shear stress/shear rates and flow rates in our channel slides.





HUVECS under perfusion

Shows how to set up a flow experiment using µ-Slide I Luer and HUVECs.



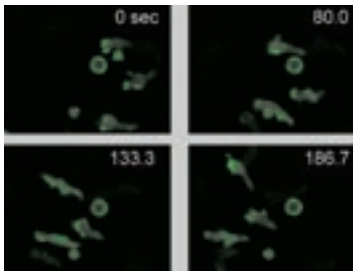
Movies

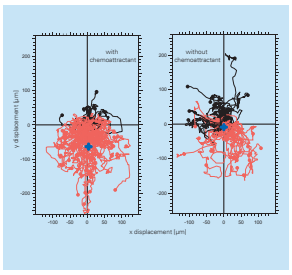
- How to prepare a chemotaxis assay in a µ-Slide
- How do cells behave under perfusion conditions
- How should chemotaxis assays be run and analyzed

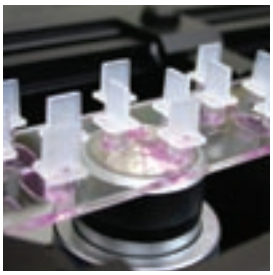
Send in your video!

2 day course at ibidi Munich / Germany

Chemotaxis and video microscopy





Aim:

The aim of the course is to learn the experimental setup and the analysis of chemotactical assays, by means of video microscopy. The focus is the analysis of adherent, slow migrating cells for example endothelial and tumor cells. Experiments are performed and analyzed using HT 1080. The main topics are: sample preparation, video microscopy of migrating cells, cell tracking, data analysis and presentation of the results. Finally, characteristic parameters are evaluated which describe directed and/ or undirected cell motion.

Schedule Day 1

Starts at 11.00 am

Welcome and icebreaker

Schedule of the training / preliminary discussion

Lunch

"Hands-on" part 1
Cell preparation / cell seeding

Talk 1: Basics of chemotaxis

"Hands-on" part 2
Setup of an experiment (measurement overnight)

Introduction to data analysis

Summary / *Wrap up at around 6 pm*

Dinner in Munich

Schedule Day 2

"Hands-on" part 3
Analysis of the data taken overnight

Introduction to tracking and analysis software

Lunch

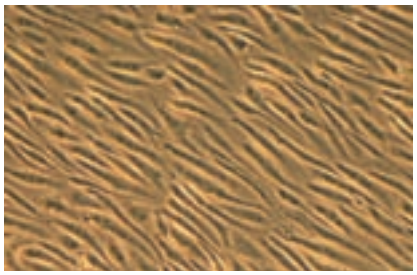
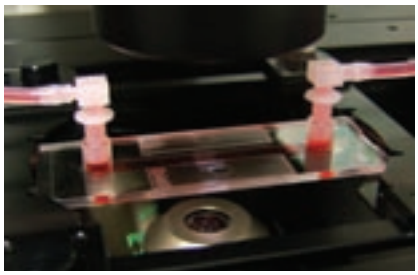
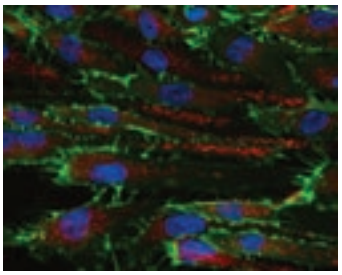
Talk 2: Chemotaxis advanced level / discussion

End of training at around 4 pm

The number of participants is limited to 4. Price per participant is 500 Euro (plus tax). The workshop is offered in either English or German. Please visit ***www.ibidi.com*** *for effective dates and course plannings. If you have any further questions please do not hesitate to contact us.*

2 day course at ibidi Munich / Germany

Perfusion based assays and video microscopy

Aim:

Cell types like endothelial cells in blood vessels face various shear stress situations *in vivo*. Such a mechanical stimulus is reflected by the physiological behaviour of *in vitro* cell cultures, if kept under flow. The aim of this course is therefore to support scientists with a profound background in cell biology to establish perfusion based assays in their lab. The course contains theoretical sections which address various flow characteristics like shear rates, viscosity, ways to create the flow etc. and practical parts where cells are seeded, cultivated and imaged under flow conditions.

Schedule Day 1

Starts at 11.00 am

Welcome and icebreaker

Schedule of the training / preliminary discussion

Lunch

Talk 1: Practical aspects of cultivation under perfusion

"Hands-on" Part 1
Cell seeding and cultivation

Talk 2: Physical basics of perfusion

"Hands-on" Part 2
Creating a flow

Summary day 1 / *Wrap up at around 6 pm*

Dinner in Munich

Schedule Day 2

"Hands-on" Part 3
Creating a movie from the overnight data

Movie analysis

Fixation and staining of cells

Lunch

Talk 3: Flow assays equipment, set-up

Discussion on individually planned experiments

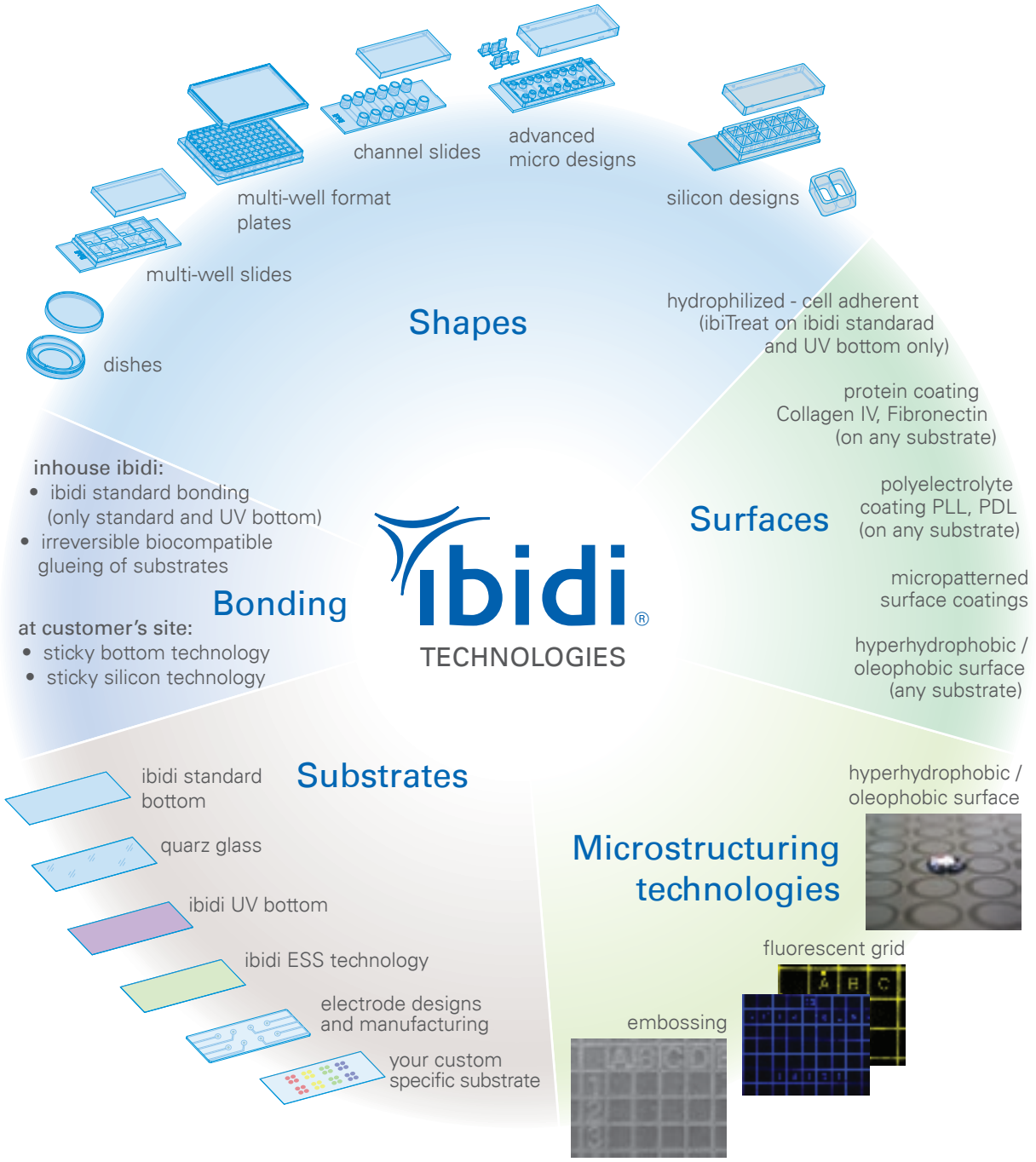
End of training at around 4 pm

both workshops include:

- hardcopies of talks
- certificate of participation
- snacks and drinks
- dinner in Munich

The number of participants is limited to 4. Price per participant is 500 Euro (plus tax). The workshop is offered in either English or German. Please visit ***www.ibidi.com*** *for effective dates and course plannings. If you have any further questions please do not hesitate to contact us.*

Technology scout



Our innovation is significantly driven by application profiles and questions from the ibidi user community. At this point we would like to encourage everybody using our products to share ideas, questions, and problems in the context of cell based assays and anything related to cell culture and microscopy. In many cases, we are able to provide economic and fast solutions for your specific questions.

If you are interested in custom specific solutions please contact us directly at customer.solutions@ibidi.com.

ibidi offers its technological solutions to academic and industrial partners. From our R&D department emerges essential know-how for designing functional disposables and instruments which address cell based assays and other applications in life sciences.

Our capabilities:

- 3D CAD design
- advanced optical injection moulding with low tolerances
- ready-to-use solutions including sterilization and packaging

Our know-how pool:

- cells in microstructures
- biocompatible materials
- microfluidics
- application consulting
- advanced surface technologies
- micropatterning
- electrodes on planar substrates
- perfusion systems
- heating setups
- electronic integrated processor based controllers

INNOVATIS

The Cedex Smart Slide was developed in cooperation with Roche Innovatis AG for the Cedex XS cell analyzer.

The Cedex XS provides fast results combined with a high image quality. In addition to cell concentration and viability, the Cedex XS also provides an analysis of cell diameter and aggregates and displays the results in histograms. Only 10 µl sample volume are required for a single analysis.

Please find order information at www.innovatis.com

Advalytix
Part of Beckman Coulter

In cooperation with Advalytix, ibidi developed a removable 12 well chamber for the Advalytix AmpliGrid system. That combination forms the new AmpliCell system, an innovative micro scale Cell Culture-to-PCR platform. Together with the AmpliSpeed instrument Advalytix provides a comprehensive solution for high quality PCR analysis in volumes as small as 1 µl.

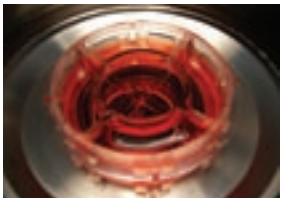
Please find order information at www.advalytix.com

Nikon

The Hi-Q4 dish was developed in cooperation with Nikon for the Nikon Biostation IM.

BioStation IM combines an incubator, a microscope and a cooled CCD camera in a compact body, allowing sophisticated time-lapse imaging, which until now has only been achieved with complex systems.

Please find order information at www.nikoninstruments.com



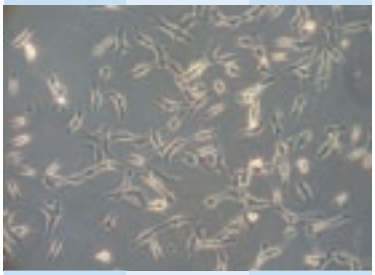
Compatibility with microscopy techniques

ibidi standard bottom	ibidi glass bottom	Standard Polystyrene plates & dishes

Phase contrast

ibidi specific comment: Phase contrast is almost not material dependent. Crucial is the meniscus which is formed at the air water interface in small open wells. Therefore channel µ-Slides are great tools for phase contrast.

Principle: Phase contrast is a technique which converts small phase shifts in cells into amplitude or intensity contrast. This label-free technique is strongly dependent on the right alignment of phase plate and annular ring in the optical pathway. It is by far the most frequently used method in biological light microscopy. A common problem in phase contrast is the meniscus formation at air liquid interfaces making this technique nearly non applicable to small culture wells.



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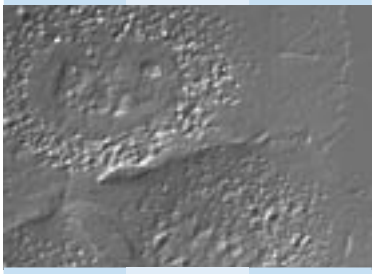
DIC – Differential Interference Contrast

ibidi specific comment: DIC is 100% compatible with the ibidi standard bottom and glass bottom. There are drawbacks in using DIC with channel slides and plastic lids. Therefore we offer special DIC lids (page 56). On request we can also produce DIC compatible channel slides.

Principle: DIC is a label free microscopy technique with high sensitivity to thin cellular material even if it is located within thick tissue. Less sensitive to meniscus formation, it has high requirements at low birefringence and is therefore not compatible with standard culture ware, manufactured of polystyrene.



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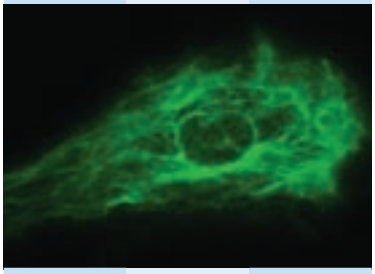
Widefield fluorescence

ibidi specific comment: The ibidi standard bottom is optimized for fluorescence microscopy, so widefield fluorescence is possible without any restriction.

Principle: Widefield fluorescence microscopy is a form of light microscopy. The specimen is illuminated with filtered light at wavelengths that excite fluorophores. It requires labeling using special antibody reactions or tagged proteins (green fluorescent protein). Fluorescence is used to detect structures, molecules or proteins within the cell up to highest magnifications.



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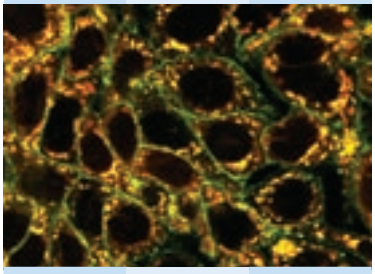
Confocal microscopy

ibidi specific comment: The ibidi standard bottom is optimized for fluorescence microscopy, so confocal microscopy is possible without any restriction.

Principle: Confocal microscopy is based on conventional widefield microscopy. Laser light is focused into the sample exciting only a small spatial area. Pinholes inside the optical pathway cut off signals being out of focus, creating images of one single optical plane. With this technique it is possible to create 3D images from data which were generated from several optical planes.



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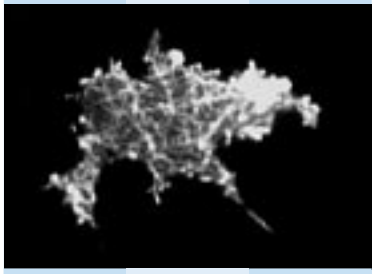
TIRF – Total Internal Reflection Fluorescence

ibidi specific comment: TIRF is possible with the ibidi standard bottom but we recommend using the glass bottom.

Principle: TIRF utilizes the evanescent field, created when a beam of light strikes an interface between two media to excite fluorescent dyes in the specimen. Although, TIRF cannot image deep into a specimen, it allows imaging of the specimen near the interface with high contrast. That technique requires two optical media with different refractive indices, such as glass (n=1.52) and water (n=1.33).



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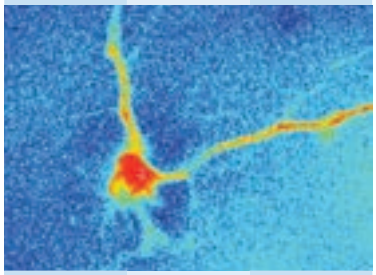
ibidi standard bottom	ibidi glass bottom	Standard Polystyrene plates & dishes



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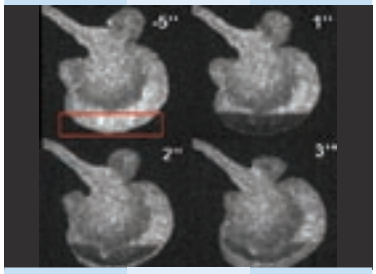

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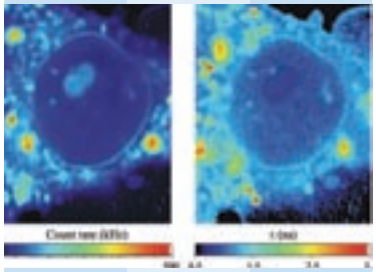

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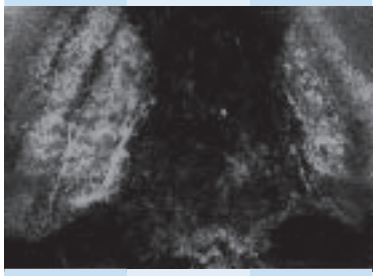

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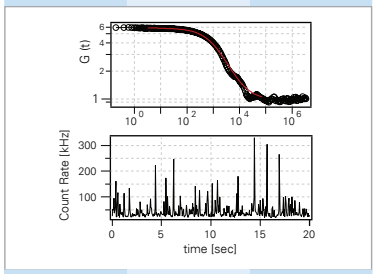

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FRET – Förster Resonance Energy Transfer

ibidi specific comment: The ibidi standard bottom is optimized for fluorescence microscopy, so FRET microscopy is possible without any restriction.

Principle: FRET is a fluorescence technique that determines the precise location and nature of the interactions between fluorophores in living cells. A donor fluorophore in its excited state can transfer its excitation energy to an acceptor fluorophore in a non-radiative fashion. This happens typically through dipole-dipole coupling in a distance less than 10 nm. Beyond that distance (Förster radius) the two fluorophores show normal fluorescence behavior.

FRAP – Fluorescence Recovery After Photobleaching

ibidi specific comment: The ibidi standard bottom is optimized for fluorescence microscopy, so FRAP is possible without any restriction.

Principle: FRAP is a fluorescence microscopy method to study the mobility of fluorescently labeled molecules. A typical FRAP experiment involves three distinct phases. After registration of the initial fluorescence, the fluorescent molecules are photobleached within a selected area using the laser beam. Afterwards, the fluorescence recovery is recorded arising from the exchange of photobleached molecules by intact ones from the immediate surroundings by diffusional or active transport .It is then possible to obtain the diffusion coefficient and a local (im)mobile fraction via modelling.

FLIM – Fluorescence Lifetime Imaging Microscopy

ibidi specific comment: The ibidi standard surface is optimized for fluorescence microscopy, so FLIM microscopy is possible without any restriction.

Principle: In contrast to normal fluorescence microscopy, where the intensity is used to create an image of the specimen, FLIM uses the lifetime of the signal by analyzing the fluorophore's exponential decay rate. By detecting differences in lifetime, it is possible to distinguish fluorophores having the same excitation and emission spectrum. The fluorescence lifetime is dependent on ion intensity, oxygen concentration, molecular binding, and molecular interaction. On the other hand, FLIM signals are independent of dye concentration, excitation light intensity, and photobleaching.

2-Photon microscopy

ibidi specific comment: The standard surface is optimized for fluorescence microscopy so 2-Photon microscopy is possible without restrictions.

Principle: 2-Photon microscopy uses the fact that a fluorophore can also be excited when it is hit at the same time (typically within several femtoseconds) by two photons. Typically the wavelength of the two photons doubles the normal excitation, so the excitation energy is added up causing a fluorescence signal. The probability of having two photons at the same time at the same spot is only given in the focal plane of high numerical aperture objective lenses. The high excitation wavelength is less phototoxic and it enhances the penetration depth when imaging thick tissue material.

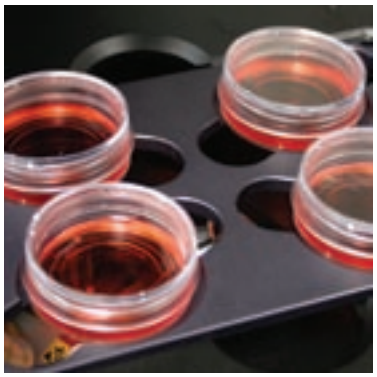
FCS – Fluorescence Correlation Spectroscopy

ibidi specific comment: FCS is only partially compatible with the ibidi standard bottom. We recommend using glass bottom versions.

Principle: FCS is a complementary technique to FRAP that is typically implemented on a laser scanning microscope. The laser beam is held stationary at a fixed location in the sample. A fluorescent molecule that moves into the confocal detection volume will cause a slight increase in fluorescence intensity. The intensity will fluctuate as fluorescent molecules move in and out of the detection. From these fluctuations an average number of molecules and their dynamic properties can be calculated. FCS is not image-based.

µ-Dish family

Petri dishes with a thin bottom for high end microscopy

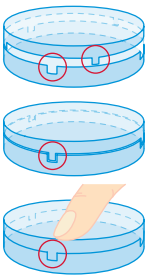


Applications

- cultivation and microscopy of cell cultures
- fluorescence microscopy of living and fixed cells
- cell manipulation combined with high resolution microscopy
- perfect cell imaging

Technical features

- standard format
- ideal cell growth with ibiTreat surface
- lid with lock position for minimized evaporation
- bottom suitable for DIC
- special DIC lids available

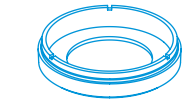


open position easy opening

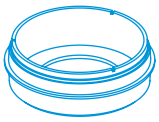
close position for cell cultivation

lock position for long term studies no evaporation

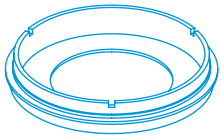
Formats



low 35 mm



high 35 mm



low 50 mm

Features

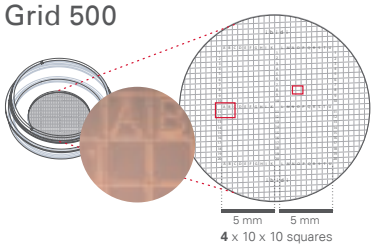
Lid

glass for DIC

standard

DIC lids for 35 mm dishes / 5 pcs. 80050

Grid 500



Surfaces

ibiTreat tissue culture treated

uncoated for self coatings

glass

ESS elastic surface

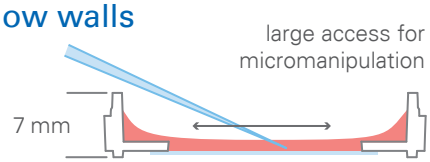
µ-Dish 35mm, low

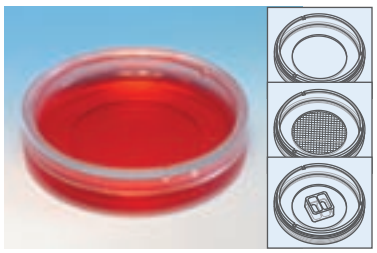
35 mm Dish with a bottom for high end microscopy and micromanipulation – low walls

Applications

- cell manipulation and microinjection
- fluorescence microscopy of living and fixed cells
- now also available with DIC lids

low walls





µ-Dish 35mm, low

ibiTreat, tissue culture treated / 60 pcs.	80136
hydrophobic, uncoated / 60 pcs.	80131

µ-Dish 35mm, low with Grid-500

ibiTreat, tissue culture treated / 60 pcs.	80156
hydrophobic, uncoated / 60 pcs.	80151

µ-Dish 35mm, low with Culture-Insert

ibiTreat, tissue culture treated / 30 pcs.	80206
hydrophobic, uncoated / 30 pcs.	80201

optional DIC lid

DIC lids for 35 mm dishes / 5 pcs.	80050

technical details

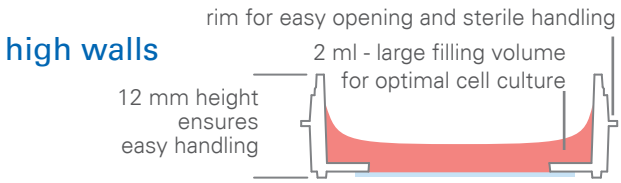
Ø µ-Dish 35mm, low	35 mm
recommended volume per µ-Dish 35mm, low	800 µl
growth area	3.5 cm^2
Ø of observation area	21 mm
height with / without lid	9/7 mm
bottom matches coverslip	No. 1.5

µ-Dish 35mm, high

35 mm Petri dish with a thin bottom for high end microscopy and cell based assays

Applications

- cultivation and microscopy of living and fixed cells
- regular cell based assays



technical details

Ø µ-Dish 35mm, high	35 mm
recommended volume per µ-Dish 35mm, high	2 ml
growth area	3.5 cm²
Ø of observation area	21 mm
height with / without lid	14/12 mm
bottom matches coverslip	No. 1.5

optional DIC lid

DIC lids for 35 mm dishes / 5 pcs.	80050

µ-Dish 35mm, high

ibiTreat, tissue culture treated / 60 pcs.	81156
hydrophobic, uncoated, sterile	81151

µ-Dish 35mm, high with Grid-500

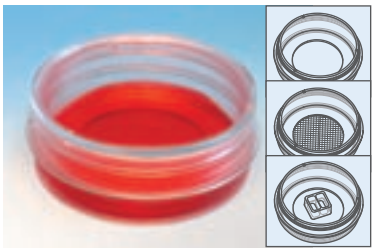
ibiTreat, tissue culture treated / 60 pcs.	81166
hydrophobic, uncoated / 60 pcs.	81161

µ-Dish 35mm, high with Culture-Insert

ibiTreat, tissue culture treated / 30 pcs.	81176
hydrophobic, uncoated / 30 pcs.	81171

µ-Dish 35mm, high with elastic surface ESS

elastic surface ESS, 28 kPa / 30 pcs.	81191
elastic surface ESS, 28 kPa coated (Collagen IV) / 30 pcs.	81192

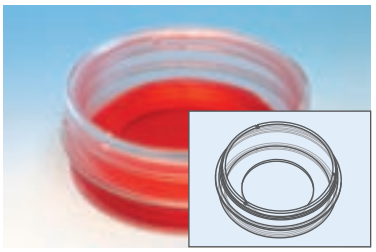


µ-Dish 35mm, high glass bottom

35 mm Petri dish with glass bottom for TIRF and single molecule applications

Applications

- cultivation and microscopy of cell cultures
- for TIRF and single molecule applications
- perfect cell imaging
- coatings might be required



technical details

see µ-Dish 35mm, high	
bottom	coverslip No. 1.5, selected quality 170 µm +/- 10 µm

catalog numbers

glass bottom dishes / 60 pcs.	81158

optional DIC lid

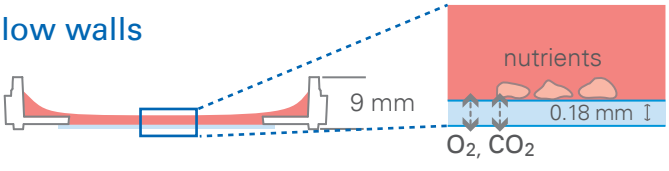
DIC lids for 35 mm dishes / 5 pcs.	80050

µ-Dish 50mm, low

50 mm Petri dish with large cultivation area and for micromanipulation

Applications

- cultivation and microscopy of cell cultures
- cultivation of large cell lawns



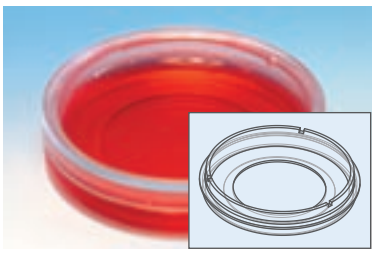
Largest growth area and volume of all ibidi products!

technical details

Ø µ-Dish 50mm, low	50 mm
volume	3 ml
growth area	7.0 cm²
Ø growth area	30 mm
height with / without lid	12 / 9 mm
bottom matches coverslip	No. 1.5

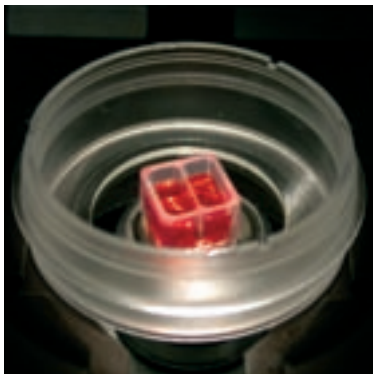
catalog numbers

ibiTreat, tissue culture treated / 30 pcs.	81136
hydrophobic, uncoated / 30 pcs.	81131



Culture-Insert family

Silicon inserts for wound healing and 2D invasion assays



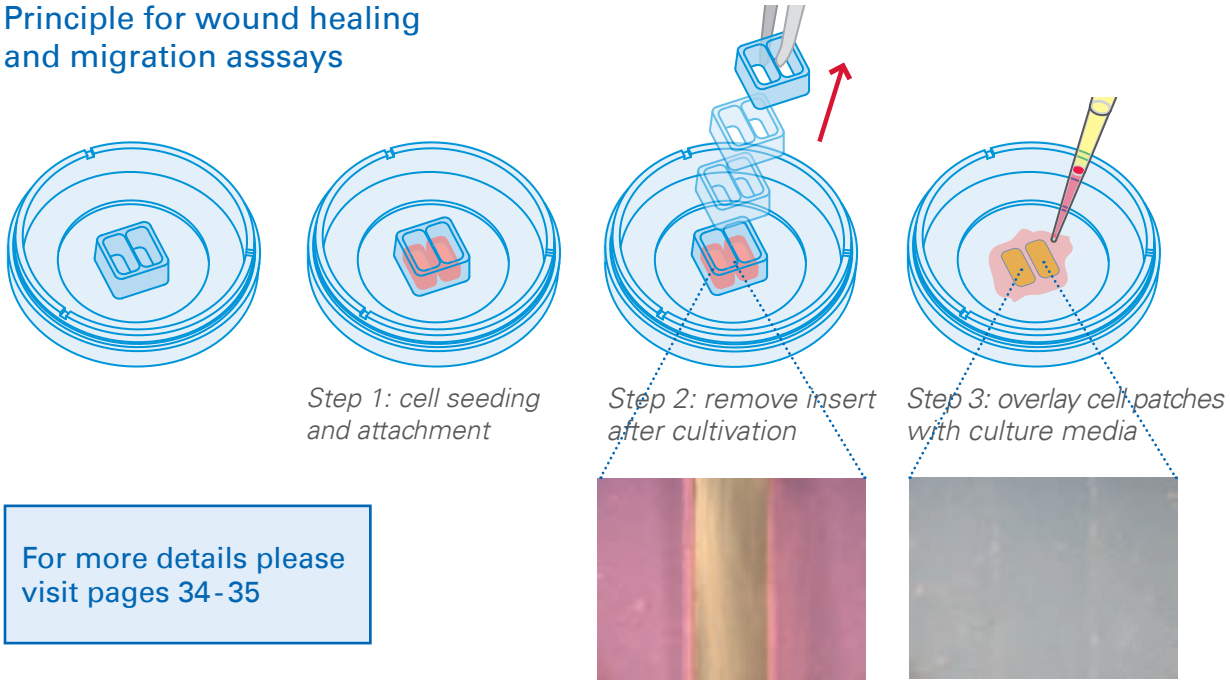
Applications

- wounding and migration assays
- co-cultivation (2D invasion assays)
- preparation of two individual cell patches
- preparation of two individual coatings

Technical features

- sticky on underside
- no material remaining when removed
- defined cell free gap of around 500 µm
- tested for cell compatibility
- small culture area ⇨ only few cells needed

Principle for wound healing and migration asssays



Step 1: cell seeding and attachment

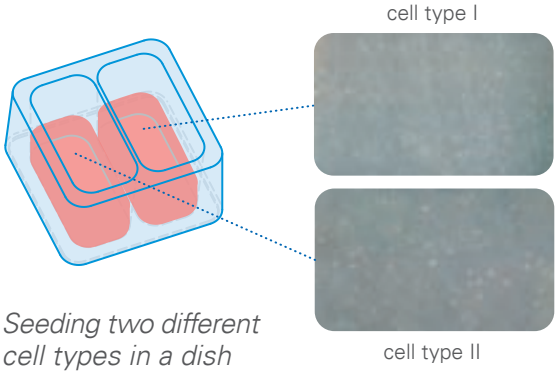
Step 2: remove insert after cultivation

Step 3: overlay cell patches with culture media

For more details please visit pages 34 - 35

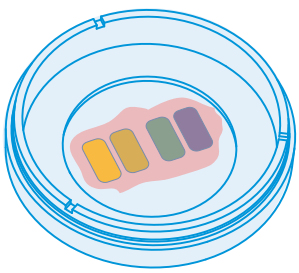
Various applications

A) 2D invasion assays



Seeding two different cell types in a dish

B) Co-cultivation

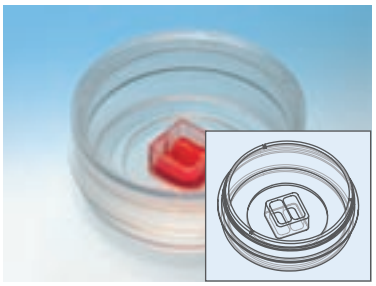


Mix multiple cell patches in a dish

Culture-Insert

Single Culture-Insert in a 35 mm µ-Dish – ready to use

- individual inserts for single experiments
- ready to use
- ideal cell growth on ibiTreat surface
- uncoated version of the µ-Dish for individual coatings and subsequent re-insertion of the Culture-Insert



technical details:

number of wells	2
outer dimensions (w x l x h) in mm	9 x 9 x 5
recommended filling volume per well	70 µl
growth area per well	0.22 cm²
width of cell free gap	500 µm +/- 50 µm

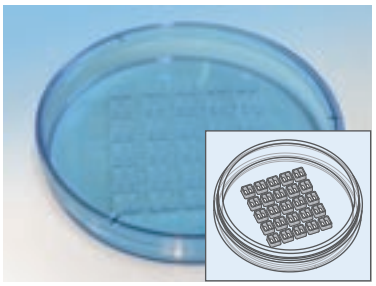
catalog numbers:

µ-Dish 35mm, low Culture-Insert, ibiTreat, sterile / 30 pcs.	80206
µ-Dish 35mm, low Culture-Insert, uncoated, sterile / 30 pcs.	80201
µ-Dish 35mm, high Culture-Insert, ibiTreat, sterile / 30 pcs.	81176
µ-Dish 35mm, high Culture-Insert, uncoated, sterile / 30 pcs.	81171

25 Culture-Inserts for self insertion

25 Culture-Inserts to be placed in your preferred experimental environment as 6 or 12 well plates

- 25 pieces in a transport dish
- for self insertion into 6 or 12 well plates or other formats
- not to be used without transfer of Culture-Inserts



technical details:

see individual Culture-Inserts

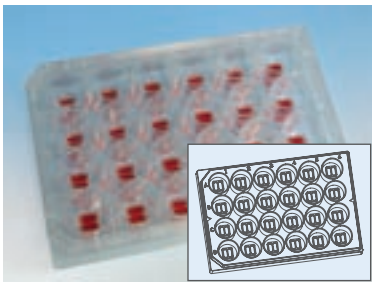
catalog numbers:

Culture-Insert, for self insertion, sterile / 1 pc.	80209

Culture-Insert 24

Culture-Inserts in a 24 well plate for screening applications

- for screening applications
- ready to use
- high parallelization
- plate made of PS – not suitable for fluorescence or high resolution microscopy



technical details:

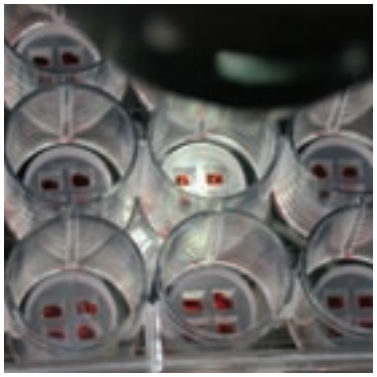
see individual Culture-Inserts

catalog numbers:

24 well plate with 24 Culture-Inserts, tissue culture treated polystyrene, sterile / 3 pcs.	80241

Culture-Insert StemCell family

4-well silicon-insert for stem cell cultivation and long term microscopy studies



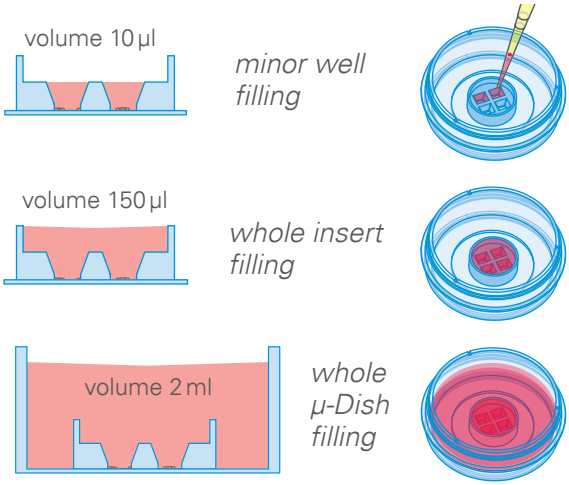
Applications

- stem cell differentiation
- long term cell microscopy
- creating patches of adherent cells or coatings
- microscopy and liquid exchange with non-adherent cells
- seeding small numbers of cells (adherent or in suspension)

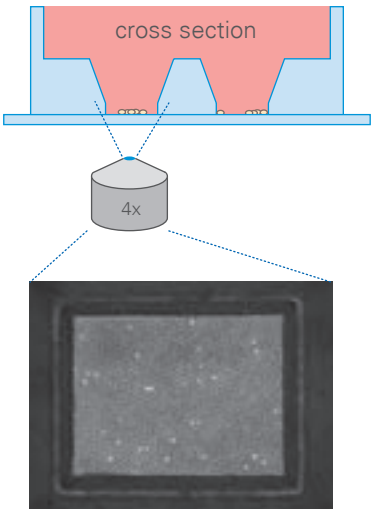
Technical features

- four small microscopy wells, 2.0 mm x 1.5 mm (digital format 4:3)
- conical wells for tracking very small cell numbers
- sticky on underside
- no material remaining when removed
- rim for easy handling with tweezers

Small number of cells large volume ⇨ low evaporation



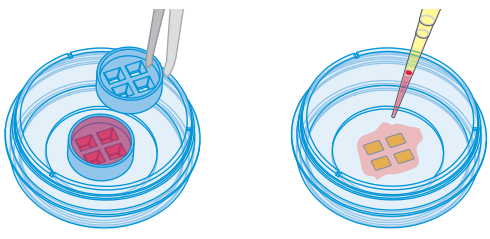
Entire well within one microscopy field



digital format 4:3

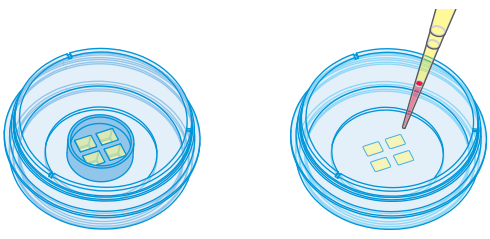
Optional: remove the Culture-Insert StemCell to create...

cell patches with adherent cells



remove the insert *fill the dish*

coating patches with proteins

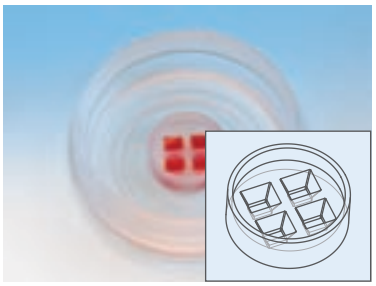


remove the insert after protein attachment *seed cells onto protein patches*

Culture-Insert StemCell

Single Culture-Insert StemCell in a 35 mm µ-Dish – ready to use

- individual inserts for single experiments
- ready to use
- ideal cell growth on ibiTreat surface
- uncoated version of the µ-Dish for suspension cells or for individual coatings and re-insertion



technical details:

number of wells	4
diameter of the complete insert	12 mm
recommended filling volume per well	10 µl
growth area per well	0.03 cm^2

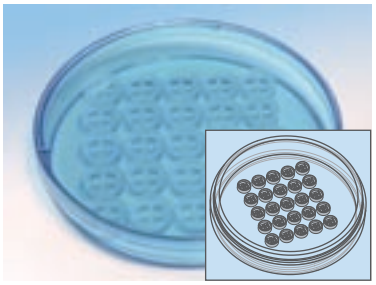
catalog numbers:

µ-Dish 35mm, high Culture-Insert StemCell, ibiTreat, sterile / 30 pcs.	80406
µ-Dish 35mm, high Culture-Insert StemCell, uncoated, sterile / 30 pcs.	80401

25 Culture-Inserts StemCell for self insertion

25 Culture-Inserts StemCell to be placed in your preferred experimental environment as 6 or 12 well plates

- 25 pieces in a transport dish
- for self insertion into 6 or 12 well plates or other formats
- not to be used without transfer of Culture-Inserts



technical details:

see individual Culture-Insert StemCell

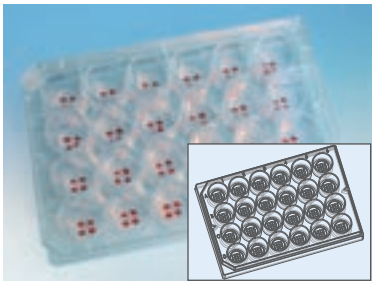
catalog numbers:

25 Culture-Insert StemCell for self insertion, sterile / 1 pc.	80409

Culture-Insert StemCell 24

Culture Inserts StemCell in a 24 well plate for screening applications

- for screening applications
- ready to use
- high parallelization
- plate made of PS – not suitable for fluorescence or high resolution microscopy
- uncoated version of the 24 well plate for suspension cells



technical details:

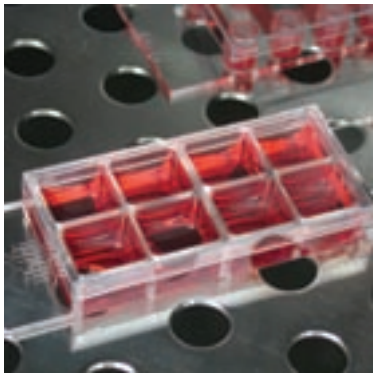
see individual Culture-Insert StemCell

catalog numbers:

24 well plate with 24 Culture-Insert StemCell, tissue culture treated polystyrene, sterile / 3 pcs.	80246
24 well plate with 24 Culture-Insert StemCell, uncoated, hydrophobic polystyrene, sterile / 3 pcs.	80245

µ-Slide 8 well

Open µ-Slide with 8 wells for immunofluorescence and high end microscopy

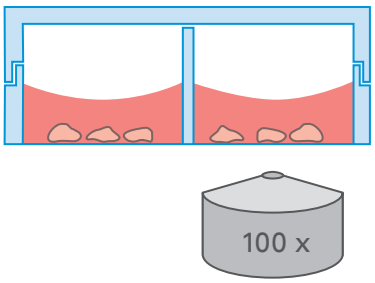


Applications

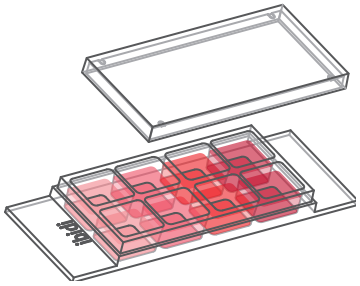
- cultivation and microscopy of cell cultures
- fluorescence microscopy of living and fixed cells
- low evaporation ⇨ long term assays

Technical features

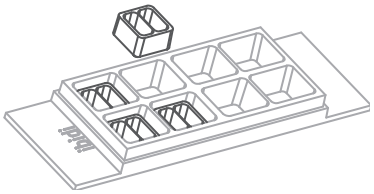
- ibiTreat surface for optimal cell adhesion
- one piece system, no peel off from the bottom
- excellent optical quality
- no glue ⇨ no leakage, no cell harm
- for staining procedures see page 27



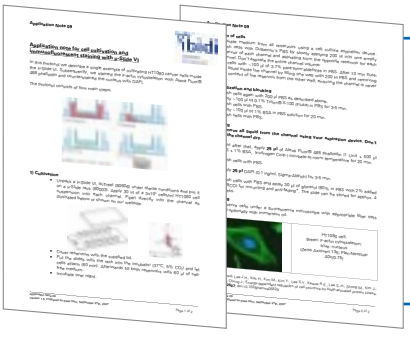
closely fitting lid to prevent evaporation



test different compound concentrations e.g. in stem cell development



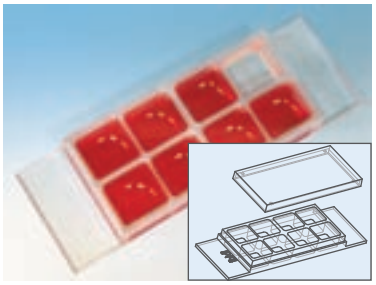
combination with Culture-Inserts possible



Detailed fluorescence staining protocols

Find detailed protocols on how to do a staining in the application section of our website: www.ibidi.com

There, all steps from the initial cell seeding, the staining, fixation and the final mounting are shown in detail.



catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	80826
coated (Collagen IV) / 15 pcs.	80822
coated (Fibronectin)* / 15 pcs.	80823
coated (Poly-L-Lysine) / 15 pcs.	80824
coated (Poly-D-Lysine)* / 15 pcs.	80825
hydrophobic, uncoated / 15 pcs.	80821

** available on request only*

technical details:

number of wells	8
dimensions of wells (w x l x h) in mm	9.4 x 10.7 x 6.8
recommended volume per well	300 µl
total height with lid	8 mm
growth area per well	1.0 cm²
bottom matches coverslip	No. 1.5

µ-Slide Angiogenesis

µ-Slide for cell microscopy on or in 3D-gel matrices, creating a flat gel surface with a minimum use of gel

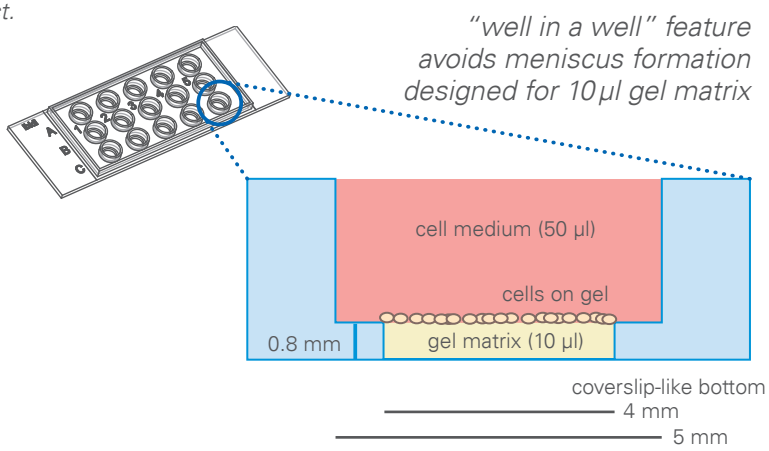
Applications

- tube formation assays
- immunofluorescence staining
- 3D cultures
- more details on page 40-41

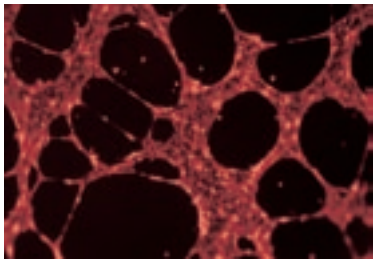
Technical features

- flat gel surface ⇨ all cells in focus
- homogeneous 0.8 mm thick gel layer
- 4 mm well in 5 mm well
- use only 10 µl of gel per well+
- low evaporation
- compatible with multi-channel pipettes

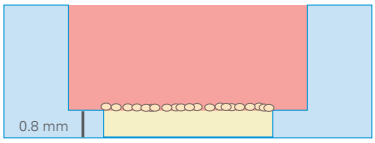
+The gel matrix is not part of the product.



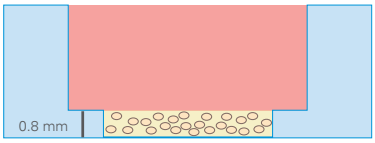
tube formation assay



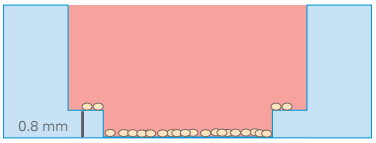
Wide range of applications



cells on gel matrix



cells in gel matrix



cells without gel matrix

technical details:

number of wells	15
volume inner well	10 µl
Ø inner well	4 mm
volume upper well	50 µl
Ø upper well	5 mm
growth area per inner well	0.125 cm²
bottom matches coverslip	No. 1.5

catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	81506
hydrophobic uncoated / 15 pcs.	81501
Microdissection, PEN-membrane / 15 pcs.	81531

µ-Slide 2x9 well

µ-Slide with 2 major chambers each with 9 subdivisons for co-cultivation assays

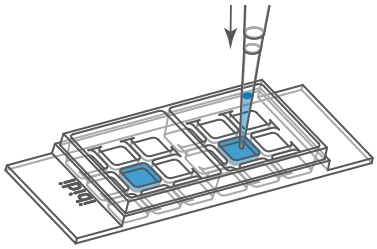
Applications

- co-culture experiments with different cell lines or primary cells
- scratch assays with co-cultures
- cultivation and microscopy of cell cultures
- *in-situ* cell experiments without cell transfer

Technical features

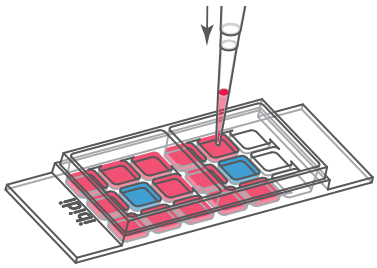
- two major wells each with 9 minor wells
- cells share soluble factors but grow separated
- low evaporation for long term assays
- excellent optical quality

Seeding cells



Step 1:

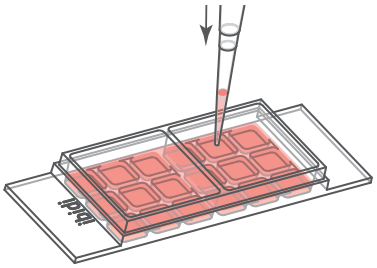
Seed 60 µl of recipent cells in the inner reservoir.



Step 2:

Seed 60 µl of the feeder cells in the outer reservoirs.

Await cell attachment; aspirate all unattached cells and media.

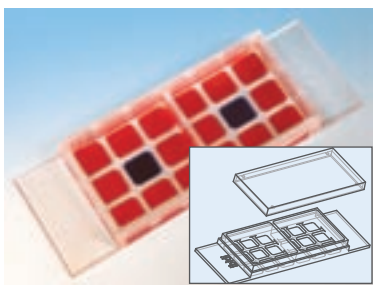


Step 3:

Overlay each major well with 600 µl cell free media.

Detailed co-cultivation protocols

Find a detailed protocol on how to perform a co-cultivation in the application section of our website: www.ibidi.com



catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	81806
coated (Collagen IV) / 15 pcs.	81802
coated (Fibronectin)* / 15 pcs.	81803
coated (Poly-L-Lysine) / 15 pcs.	81804
coated (Poly-D-Lysine)* / 15 pcs.	81805
hydrophobic, uncoated / 15 pcs.	81801

** available on request only*

technical details:

number of major wells	2
volume per major well	600 µl
dimensions of major wells (w x l x h) in mm	21.5 x 23.6 x 6.8
number of minor wells	2 x 9
volume of each minor well	70 µl
dimensions of minor wells (w x l x h) in mm	6.1 x 6.8 x 1.3
growth area per minor well	0.4 cm^2
bottom matches coverslip	No. 1.5

µ-Slide 18 well – flat

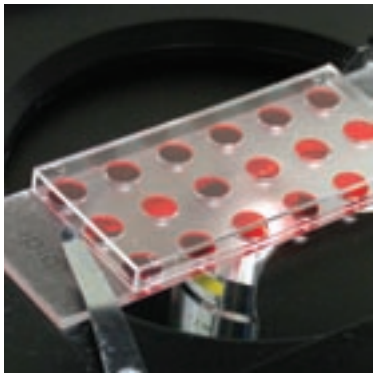
µ-Slide with 18 wells for matrix tests or spotting samples such as RNA assays

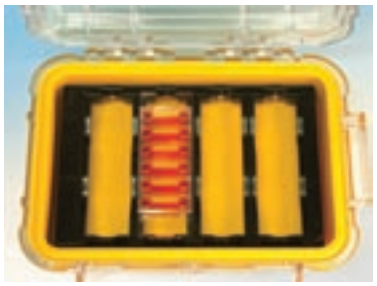
Applications

- quick immunofluorescence stainings of adherent cells
- optimization of surface functionalizations
- fast toxicological screening of small microscopy samples
- 18 fold sample preparation

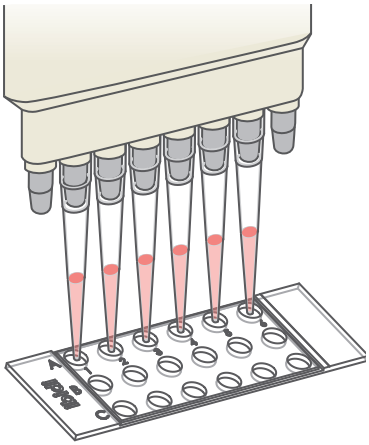
Technical features

- compatible with multichannel pipettes
- wells indicated by letters and numbers (A-C; 1-6)
- suitable for fluorescence scanners
- excellent optical quality
- very small open volume, high evaporation ⇨ suitable for short-term experiments only

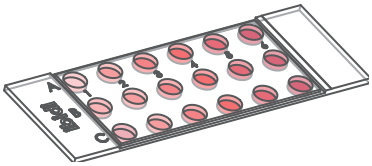




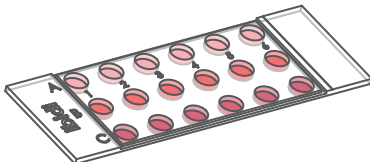
for extended assay periods combine with Olaf (see page 91)



Fully compatible with multipipettes (detach the two outer pipette tips)



For multiple immunofluorescence stainings or toxicological screenings



To test cell attachment, coat the surfaces with different cell adhesion factors, followed by cell seeding and incubation.

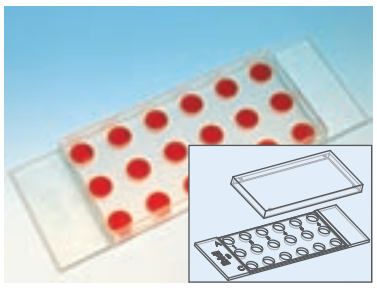
Also with a 1 µm foil for Laser Microdissection applications

technical details:

number of wells	18
recommended volume per reservoir	30 µl
well diameter	5 mm
growth area per well	0.2 cm^2
bottom matches coverslip	No. 1.5

catalog numbers:

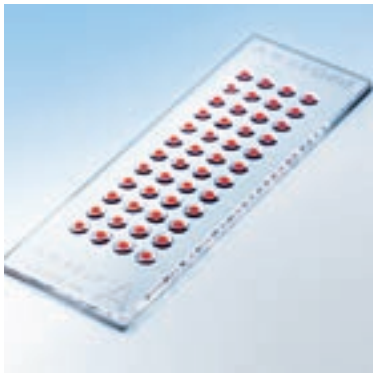
ibiTreat, tissue culture treated / 15 pcs.	81826
coated (Collagen IV) / 15 pcs.	81822
coated (Fibronectin)* / 15 pcs.	81823
coated (Poly-L-Lysine) / 15 pcs.	81824
coated (Poly-D-Lysine)* / 15 pcs.	81825
hydrophobic, uncoated / 15 pcs.	81821
Microdissection, PEN-membrane / 15 pcs.	81831



** available on request only*

AmpliCell: Cell Culture-to-PCR

12 well cell culture and PCR slide for multiple *in situ* assays



Applications

- on-slide cell culture of adherent cells and cells in solution
- *in-situ* cell experiments to PCR in few simple steps
- 4 PCR replicates per cell culture well

Technical features

- no cell preparation needed ⇨ minimized cellular stress
- full visual control of workflow
- save up to 90% reagent costs
- compatible to HT systems (e.g., FACS™, robotics)

Convenient workflow:

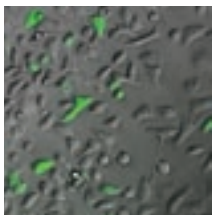
Cell culture

- *seed cells in 200-300 µl media*
- *incubate*

Cell treatment

apply assay for:

- *transfection*
- *methylation*
- *microRNA*
- *siRNA*

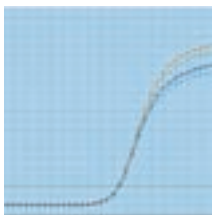


Optical control

- *remove growth chamber*
- *optically verify cultivation and treatment success*

Amplification

- *add reaction mix*
- *start AmpliSpeed slide cycler*



Analysis

analyze sample

- *gel*
- *qPCR*
- *capillary electrophoresis*

More details at www.advalytix.com/amplicell and www.advalytix.com/zell

For ordering information on the instrument "AmpliSpeed slide cycler" please contact info@advalytix.com.

FACS™ is a trademark of Becton, Dickinson and Company

catalog numbers:

5pcs/pck, Fibronectin coated	OAX04531
15pcs/pck, Fibronectin coated	OAX04532
5pcs/pck, Collagen IV coated*	OAX04533
15pcs/pck, Collagen IV coated*	OAX04534
5pcs/pck, Poly-L-Lysin coated*	OAX04535
15pcs/pck, Poly-L-Lysin coated*	OAX04536

** available on request only*

technical details:

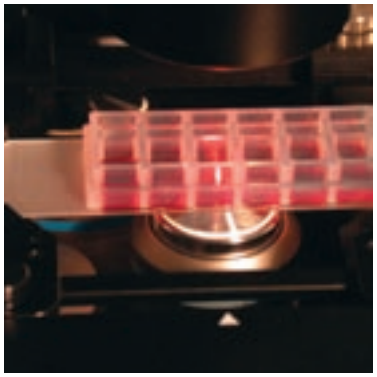
number of cultivation wells	12
max. volume per well	450 µl
dimensions of wells in mm	7.5 x 7.5 x 8
glass slide size in mm	75.6 x 25 x 1.0
number of PCR reactions	48
diameter of reaction sites	1.6 mm

µ-Chamber 12 well

Removable silicone cultivation chamber for cell culture and immunofluorescence stainings

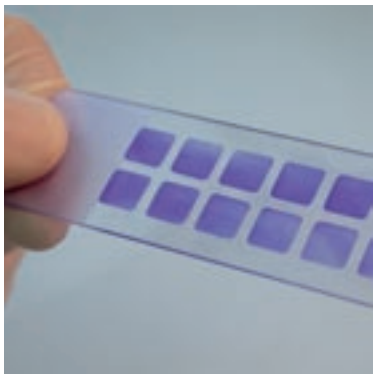
Applications

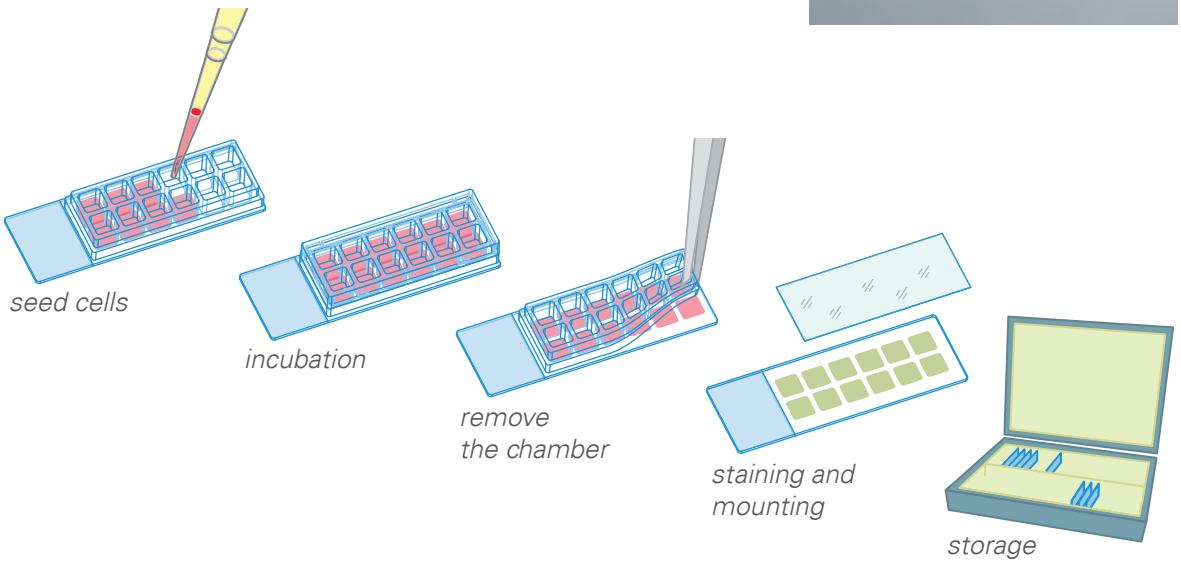
- 12 fold sample preparation
- easy immunofluorescence staining
- for use of standard staining and mounting technique with coverslip sealing



Technical features

- self-adhesive 12 well silicone gasket mounted on standard glass slide
- biocompatible silicon material
- non-fluorescent slide with frosted ends for easy handling and labeling
- no adhesive remains on slide after chamber is removed
- optional: transfer to any flat and clean surface for cell cultivation
- suitable for upright microscopes after staining and mounting with a coverslip
- long term storage of microscopy samples



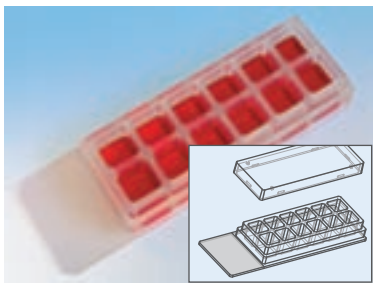


technical details:

number of wells	12
dimensions of wells (w x l x h) in mm	7.5 x 7.5 x 8
recommended volume per well	250 µl
growth area per well	0.56 cm²
bottom:	glass slide (26 x 76 x 1 mm)

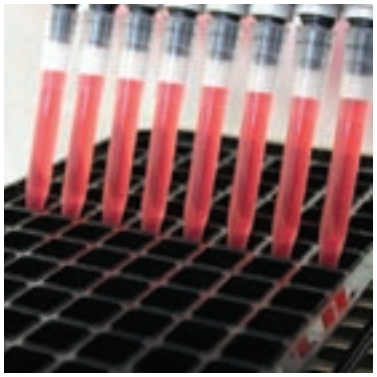
catalog numbers:

µ-Chamber 12 well, sterile, on glass slide / 15 pcs.	81201
suitable glass coverslips, unsterile 24 x 60 mm, No. 1.5 / 100 pcs.	10811



µ-Plate family

Black multi-well plates for high throughput applications and cell based assays



black material for low well-to-well crosstalk using fluorescence microscopy

Applications

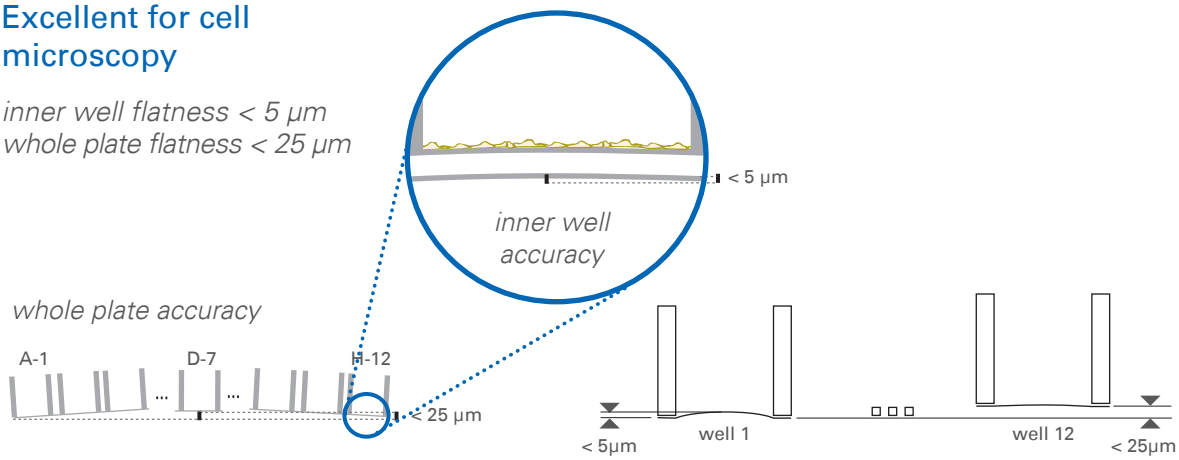
- fluorescence based imaging of living or fixed cells
- high-throughput screenings (HTS) in cell culture
- compound screenings (toxicology)
- large-scale transfection experiments

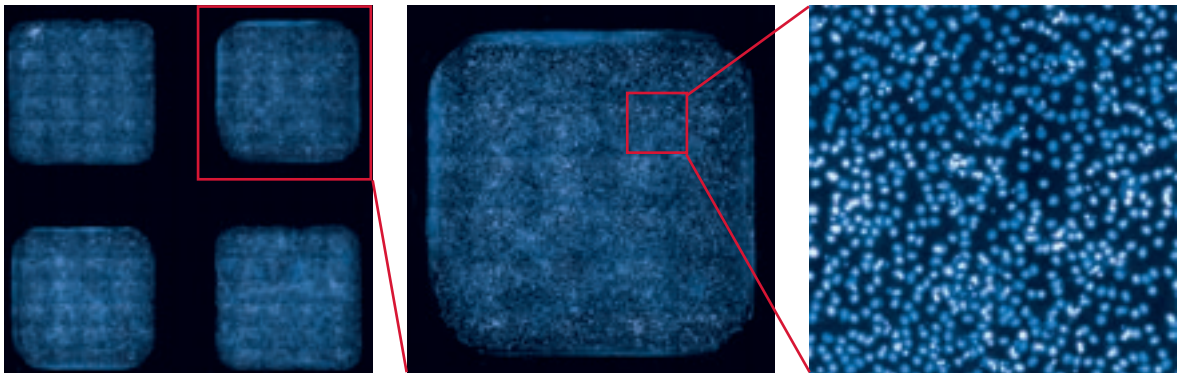
Technical features

- compatible with automation equipment
- standard format and dimensions of 85.5 x 127.5 mm (meets ANSI/SBS Standards)
- 96/384 quadratic wells with standard numbering
- excellent inner well flatness and whole plate flatness
- suitable for fluorescence scanners
- high quality plastic bottom (coverslip-like #1.5)
- compatible with solvents for staining and fixation
- compatible with immersion oil
- sterile, single packaging

Excellent for cell microscopy

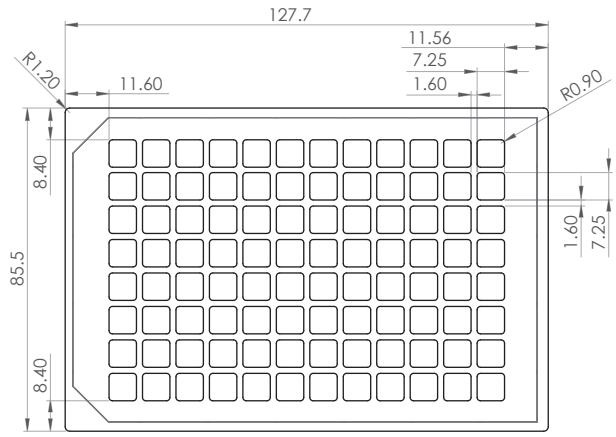
inner well flatness < 5 µm
whole plate flatness < 25 µm

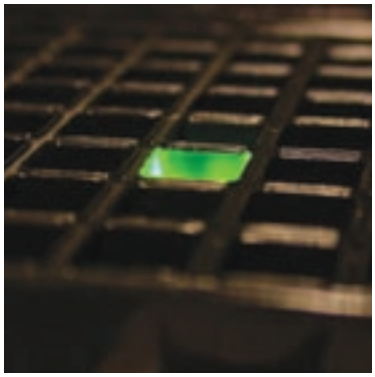




µ-Plate 96 well

96 quadratic wells for medium throughput with flat and clear bottom





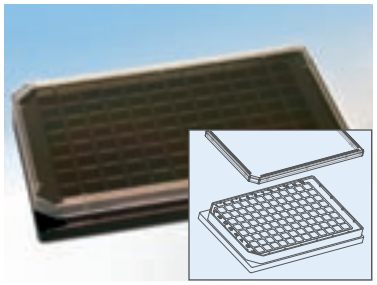
technical details:

length / width	127.7 / 85.5 mm
height with /without lid	17.2 / 15.0 mm
single well depth	13.0 mm
well to well distance	9.1 mm
single well dimensions	7.3 x 7.3 mm
single well growth area	0.55 cm²
single well coating area using 300 µl	2.35 cm²

inner well flatness	± 5 µm
whole plate flatness	± 25 µm
bottom matches coverslip	No. 1.5

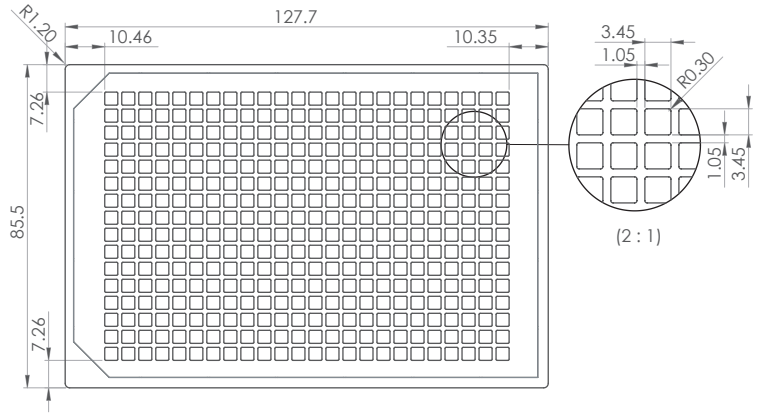
catalog numbers:

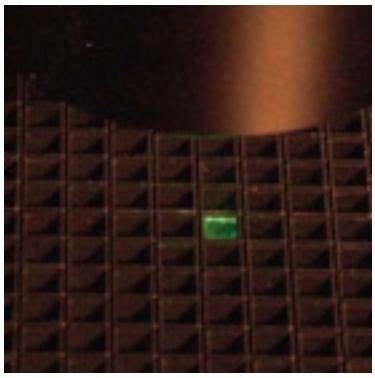
ibiTreat, tissue culture treated / 15 pcs.	89626
hydrophobic, uncoated / 15 pcs.	89621



µ-Plate 384 well

384 quadratic wells for high throughput applications in cell based assays





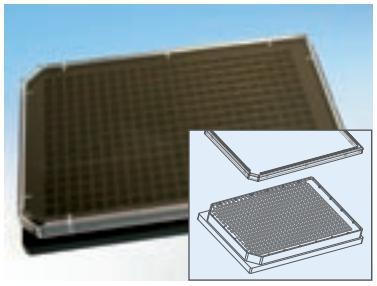
technical details:

length / width	127.7 / 85.5 mm
height with /without lid	17.2 / 15.0 mm
single well depth	12.7 mm
well to well distance	4.55 mm
single well dimensions	3.4 x 3.4 mm
single well growth area	0.11 cm²
single well coating area using 50 µl	0.80 cm²

inner well flatness	± 5 µm
whole plate flatness	± 25 µm
bottom matches coverslip	No.1.5

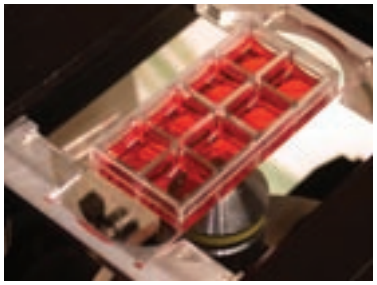
catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	88406
hydrophobic, uncoated / 15 pcs.	88401



sticky-Slide family

Slides without any bottom but with self-adhesive underside for attaching your own substrate



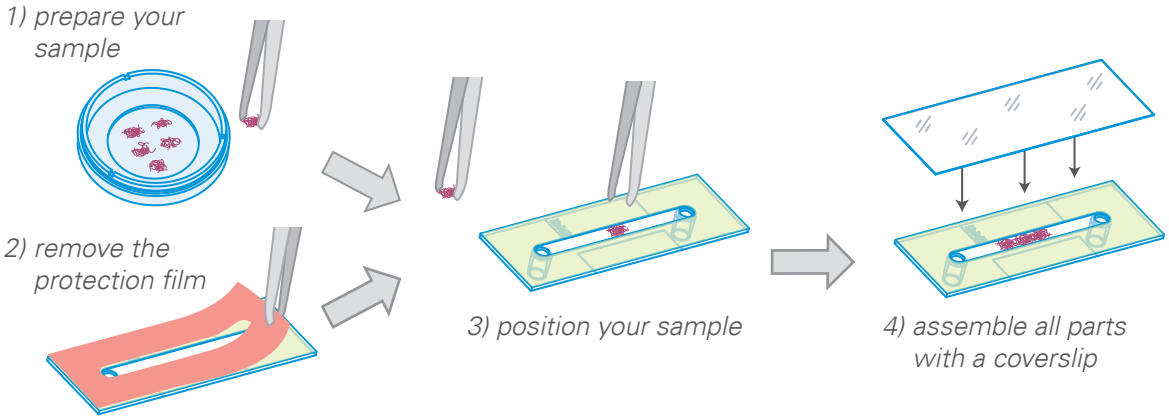
Applications

- housing for several cell culture applications
- insertion of materials or tissue into perfusion channels
- use of specific bottom materials, like plastic sheets, silicon chips and slides

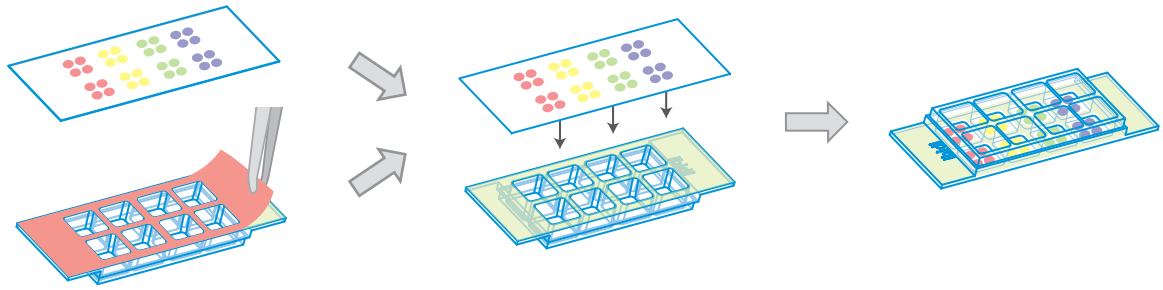
Technical features

- self-adhesive underside
- biocompatible adhesive (cell culture tested)
- slides come without bottom material, sterile packed
- sticky on all flat surfaces; even wet surfaces
- suitable sterile coverslips available

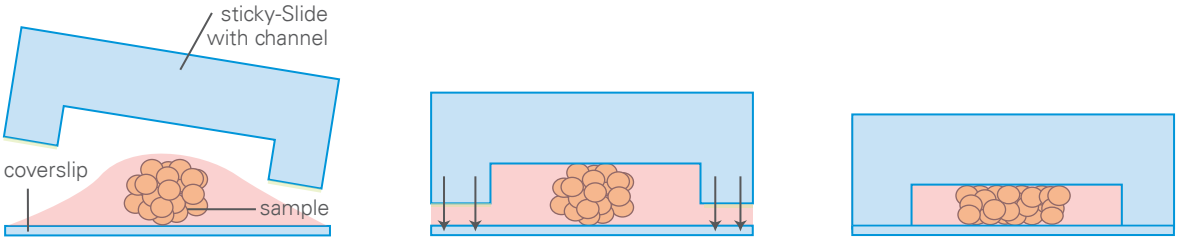
Example setup I – sample for perfusion



Example setup II – spotted coverslip

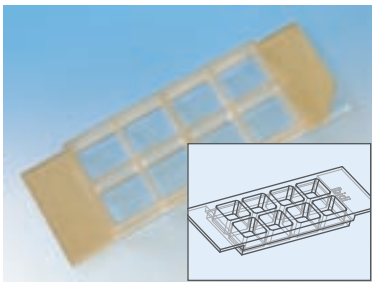


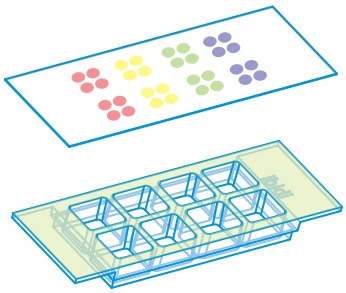
Example setup III – squeezed sample in channel



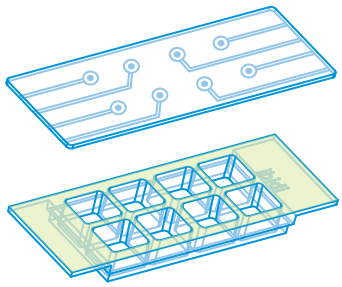
sticky-Slide 8 well

Technical details identical to µ-Slide 8 well but **without** bottom

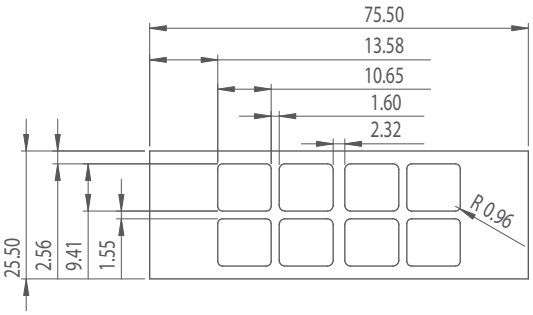




housing of spotted coverslips



housing of circuit boards



technical details:

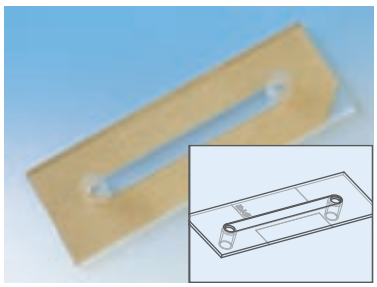
number of wells	8
dimensions of wells (w x l x h) in mm	9.4 x 10.7 x 6.8
recommended volume per well	300 µl
total height with lid	8 mm
growth area per well	1.0 cm²
bottom	none

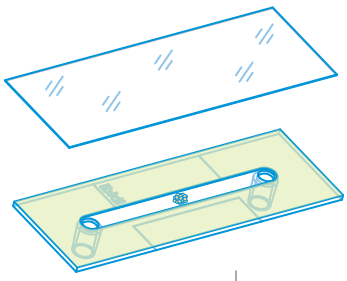
catalog numbers:

sticky-Slide 8 well, sterile / 15 pcs.	80828
glass coverslips, sterile 25 x 75 mm, No. 1.5 / 100 pcs.	10801

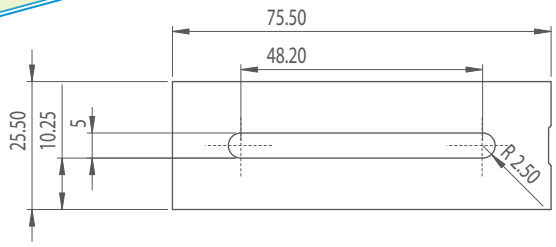
sticky-Slide I Luer

Technical details identical to µ-Slide I Luer but **without** bottom





cells or tissue in channels for perfusion



technical details: µ-Slide I	0.1 Luer	0.2 Luer	0.4 Luer	0.6 Luer	0.8 Luer
volume of the channel	25 µl	50 µl	100 µl	150 µl	200 µl
height of the channel	100 µm	200 µm	400 µm	600 µm	800 µm
channel length	50 mm for all types				
bottom	none				

catalog numbers:

sticky-Slide I 0.1 Luer, sterile / 15 pcs.	81128
sticky-Slide I 0.2 Luer, sterile / 15 pcs.	80168
sticky-Slide I 0.4 Luer, sterile / 15 pcs.	80178
sticky-Slide I 0.6 Luer, sterile / 15 pcs.	80188
sticky-Slide I 0.8 Luer, sterile / 15 pcs.	80198
glass coverslips, sterile 25 x 75 mm, No. 1.5 / 15 pcs.	10801

µ-Slide I

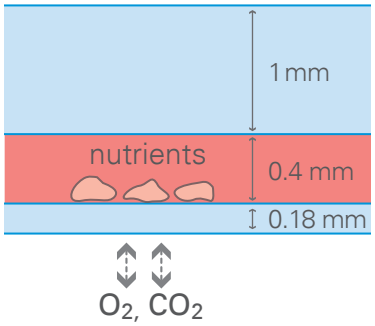
Combination of a cell culture chamber and a coverslip for imaging inside a channel

Applications

- immunofluorescence stainings with easy liquid exchange
- real-time imaging during staining process
- high resolution microscopy of living and fixed cells

Technical features

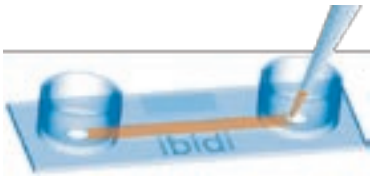
- large observation area for microscopy
- 100 µl channel ⇨ small volume
- lids for long term cultivation



Seeding cells

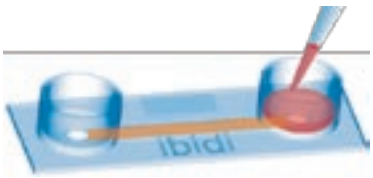
Step 1:

Fill the channel with 100 µl cell suspension



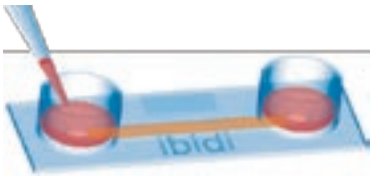
Step 2:

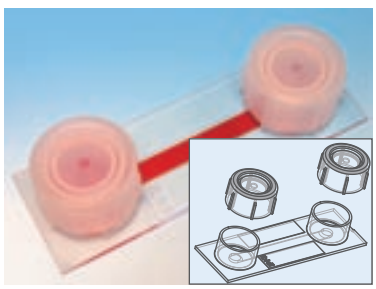
Fill one reservoir with cell-free media; surface tension prevents flushing the cells from the channel



Step 3:

Fill second reservoir with the same amount of cell-free media to equilibrate the system





catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	80106
coated (Collagen IV) / 15 pcs.	80101
coated (Fibronectin)* / 15 pcs.	80102
coated (Poly-L-Lysine) / 15 pcs.	80110
coated (Poly-D-Lysine)* / 15 pcs.	80115
hydrophobic, uncoated / 15 pcs.	80111

** available on request only*

technical details:

recommended volume per reservoir	600 µl
number of channels	1
volume of the channel	100 µl
height of the channel	0.4 mm
length of the channel	50 mm
width of the channel	5 mm
growth area	2.5 cm^2
bottom matches coverslip	No. 1.5

µ-Slide I Luer family

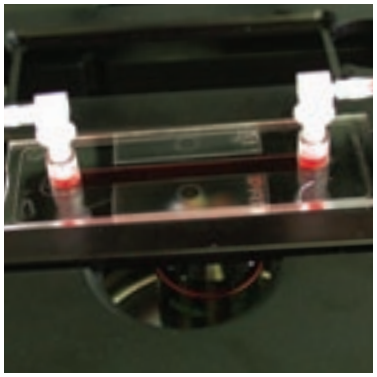
µ-Slide I Luer family for easy flow applications in a channel

Applications

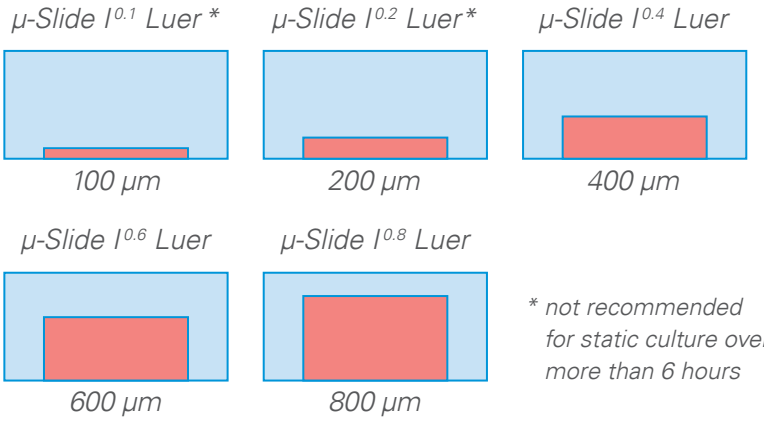
- flow applications and static cultivation
- high resolution microscopy of living and fixed cells

Technical features

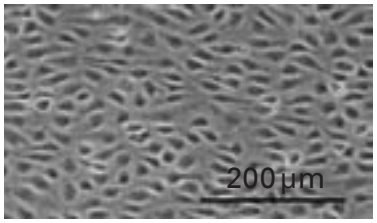
- large observation area for microscopy
- channel volume of 25 µl / 50 µl / 100 µl / 150 µl / 200 µl
- easy connection to existing tubes and pumps via Luer adapters
- available as variety pack containing all heights (3 each)
- available as flow kits (see page 82)



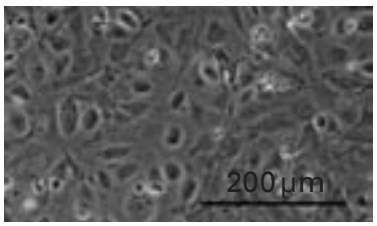
Cross section of the channel: same slide - different channel height



* not recommended for static culture over more than 6 hours



flow cultivation



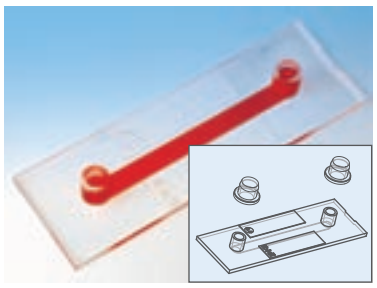
static cultivation

For detailed information on flow rates, shear stress, and shear rates visit www.ibidi.com

catalog numbers: µ-Slide I	0.1 Luer	0.2 Luer	0.4 Luer	0.6 Luer	0.8 Luer
ibiTreat, tissue culture treated / 15 pcs.	81126	80166	80176	80186	80196
coated (Collagen IV) / 15 pcs.	81122	80162	80172	80182	80192
coated (Fibronectin)* / 15 pcs.	81123	80163	80173	80183	80193
coated (Poly-L-Lysine) / 15 pcs.	81124	80164	80174	80184	80194
hydrophobic, uncoated / 15 pcs.	81121	80161	80171	80181	80191
variety pack / 15 pcs. / 3 each	ibiTreat:	81106		uncoated:	81101

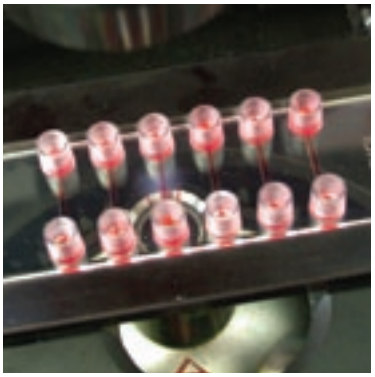
technical details:					
volume of the channel	25 µl	50 µl	100 µl	150 µl	200 µl
height of the channel	100 µm	200 µm	400 µm	600 µm	800 µm
bottom	matches coverslip No. 1.5				

* *available on request only*



µ-Slide VI 0.1

6 channel µ-Slide for flow assays with minimal volume



Applications

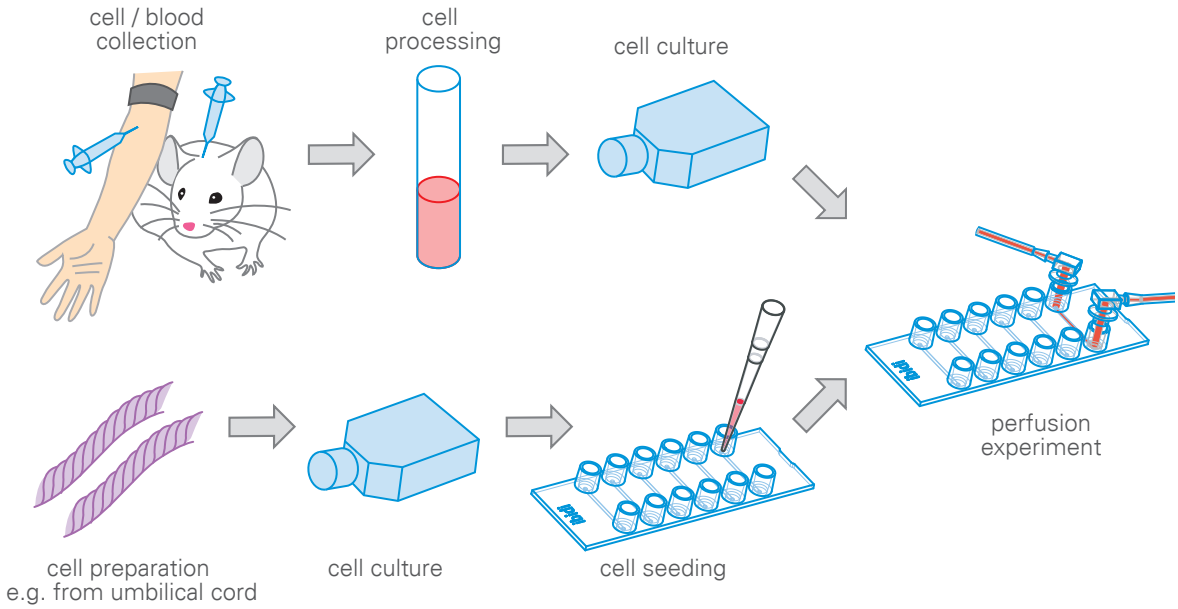
- flow assays with a minimum of cells and/or liquids (mouse model)
- parallel shear stress applications

Technical features

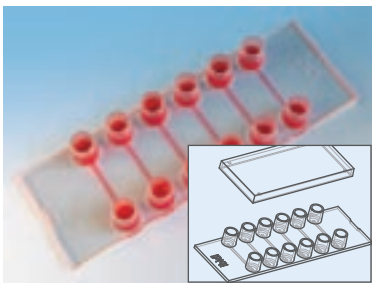
- smallest ibidi channel slide with highest shear stress
- easy connection to tubes and pumps via female Luer adapter
- available as flow kit (see page 83)
- defined shear stress and shear rate analysis+
- not for use in static cultures

+ for detailed calculations check: www.ibidi.com

Adhesion assay



For detailed protocols on immunoflurescence and flow assays please visit www.ibidi.com



catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	80666
coated (Collagen IV) / 15 pcs.	80662
coated (Fibronectin)* / 15 pcs.	80663
coated (Poly-L-Lysine) / 15 pcs.	80664
coated (Poly-D-Lysine)* / 15 pcs.	80665
hydrophobic, uncoated / 15 pcs.	80661

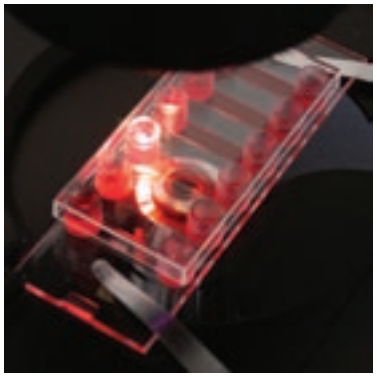
** available on request only*

technical details:

adapters	female Luer
recommended volume per reservoir	60 µl
number of channels	6
volume of each channel	1.7 µl
height of channels	0.1 mm
length of channels	17 mm
width of channels	1 mm
growth area per channel	0.17 cm^2
bottom matches coverslip	No. 1.5

µ-Slide VI 0.4

6 channel µ-Slide for multiple immunofluorescence staining and optimal phase contrast



Applications

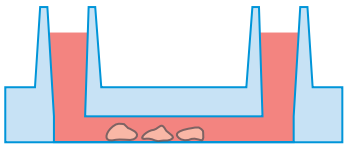
- immunofluorescence assays and live cell imaging
- real-time imaging under static or flow conditions
- parallel screenings using multichannel pipettes

Technical features

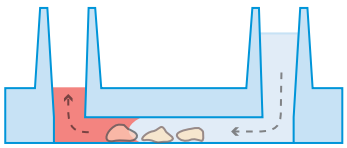
- 30 µl channel volume ⇨ saves reagents
- easy connection to existing tubes and pumps via female Luer adapter
- available as flow kit (see page 83)
- defined shear stress and shear rate analysis+

+ for detailed calculations check: www.ibidi.com

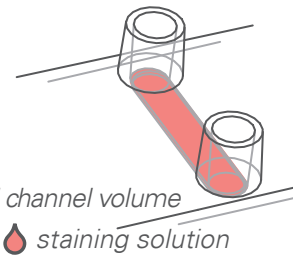
µ-Slide all-in-one chamber

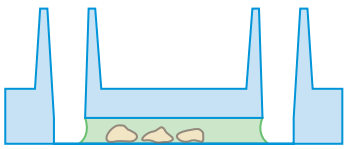


1) cultivation

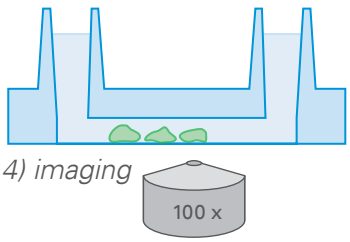


2) fixation





3) staining



4) imaging

For detailed protocols on immunoflurescence and flow assays please visit www.ibidi.com

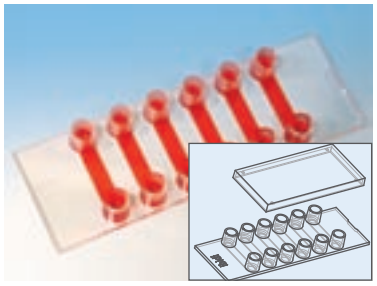
technical details:

adapters	female Luer
recommended volume per reservoir	60 µl
number of channels	6
volume of each channel	30 µl
height of channels	0.4 mm
length of channels	17 mm
width of channels	3.8 mm
growth area per channel	0.6 cm²
bottom matches coverslip	No. 1.5

catalog numbers:

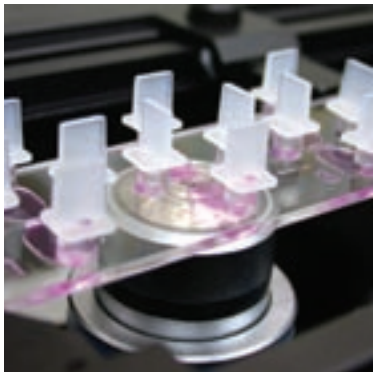
ibiTreat, tissue culture treated / 15 pcs.	80606
coated (Collagen IV) / 15 pcs.	80602
coated (Fibronectin)* / 15 pcs.	80603
coated (Poly-L-Lysine) / 15 pcs.	80604
coated (Poly-D-Lysine)* / 15 pcs.	80605
hydrophobic, uncoated / 15 pcs.	80601

** available on request only*



µ-Slide Chemotaxis

Investigate chemotaxis and migration of adherent cells in linear gradients



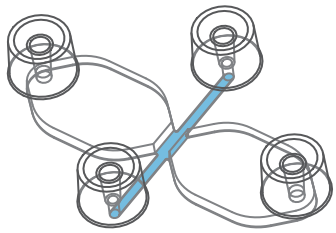
Applications

- chemotaxis of cancer, endothelial cells, fibroblasts ...
- chemotaxis of slow migrating, adherent cells
- migration assays with simultaneous cell tracking by (fluorescence) microscopy
- more details on pages 36–39

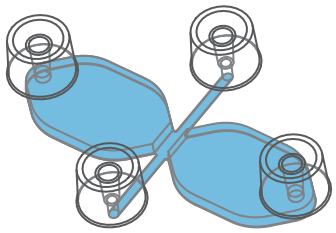
Technical features

- 3 chambers on one slide for parallel assays
- ready to use system, no assembling
- linear gradients, stable for over 48 hours
- made for high-end video microscopy

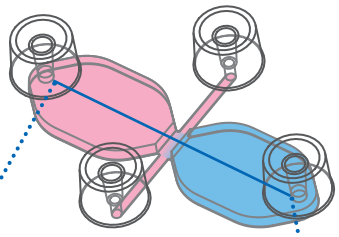
Preparation



seed cells in the cross channel



fill both reservoirs with cell free medium

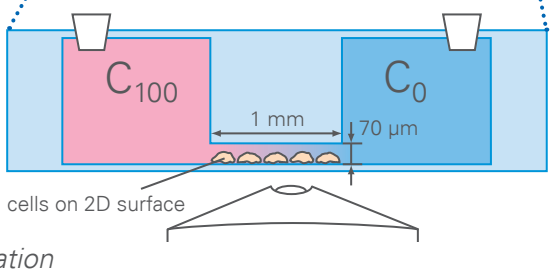


fill one of the reservoirs with chemoattractant

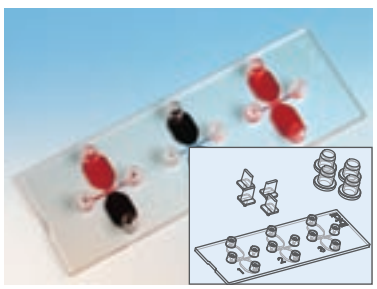
Basic principle

Two large reservoirs of 40 µl are connected by a narrow observation area.

The adherent cells inside the observation area become super-imposed by a linear and time-stable gradient.



Please visit www.ibidi.com *for a detailed application note, a handling video, and a chemotaxis analysis tool*



catalog numbers:

ibiTreat, tissue culture treated / 10 pcs.	80306
coated (Collagen IV) / 10 pcs.	80302
hydrophobic, uncoated / 10 pcs.	80301

technical details:

chemotaxis chambers on slide	3
volume per chamber	80 µl
observation area	2x1 mm²
total height with plugs	12 mm
volume chemoattractant	18 µl
bottom matches coverslip	No. 1.5

µ-Slide Chemotaxis 3D

Investigate chemotaxis and migration of non-adherent cells in gel matrices

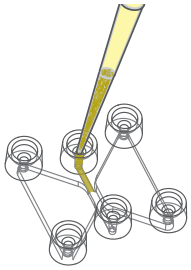
Applications

- chemotaxis of neutrophiles, lymphocytes, macrophages
- chemotaxis experiments for fast or slow migrating cells
- invasion assays with 3D gels and migrating cells

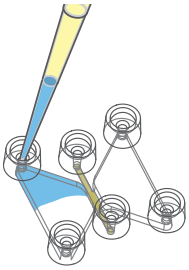
Technical features

- chamber geometry optimized for suspension cells in 3D matrices
- 3 chambers on one slide for parallel working
- ready to use system, no assembling
- chambers and reservoirs with letters and numbers

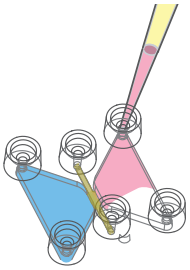
Preparation



seed cells with gel matrix



fill with chemoattractant free medium

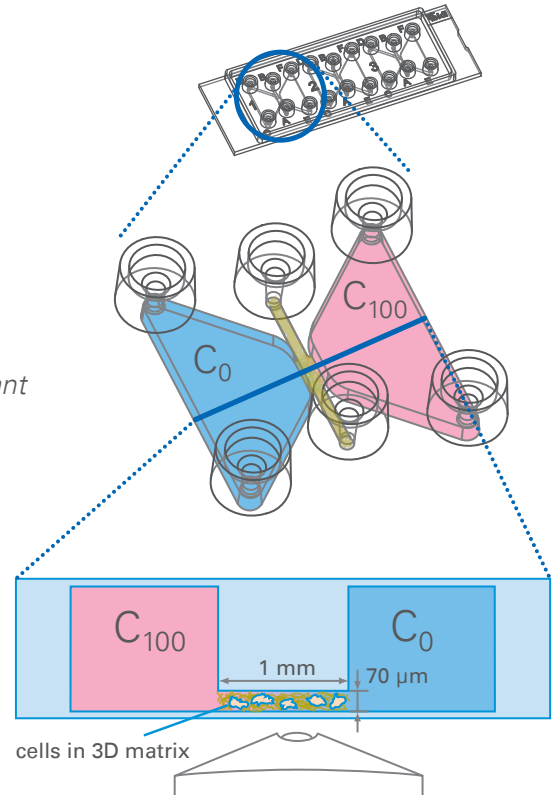


fill with chemoattractant

Basic principle

Two large reservoirs of 60 µl are connected by a narrow observation area.

The cells embedded in a gel inside the observation area become super-imposed by a linear and time-stable gradient.

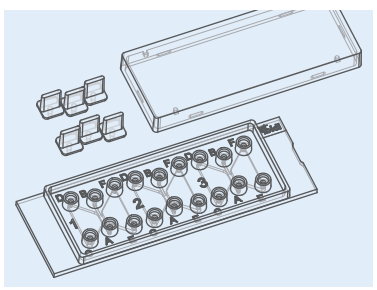


technical details:

chemotaxis chambers on slide	3
volume per chamber	120 µl
observation area	2x1 mm²
total height with plugs	12 mm
volume chemoattractant	60 µl
bottom matches coverslip	No.1.5

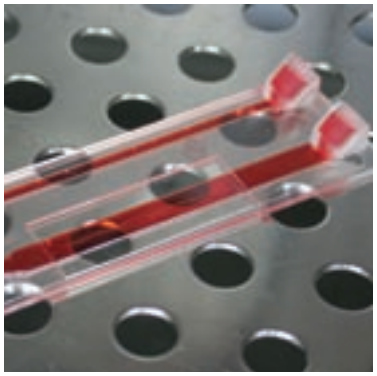
catalog numbers:

ibiTreat, tissue culture treated / 10 pcs.	80326
coated (Collagen IV) / 10 pcs.	80322
hydrophobic, uncoated / 10 pcs.	80321



µ-Slide upright 0.7

µ-Slide that addresses upright microscopy, applicable in static or perfusion assays



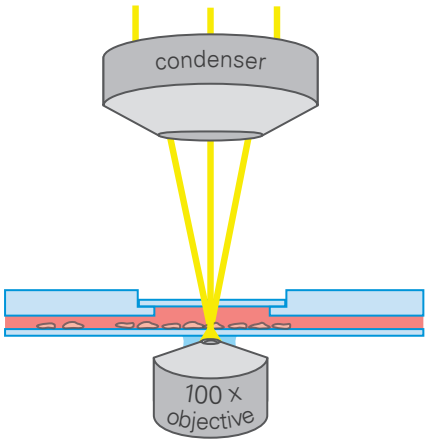
Applications

- cell culture and microscopy, using upright or inverted microscopes
- high resolution microscopy of living and fixed cells
- flow assays with upright microscopes

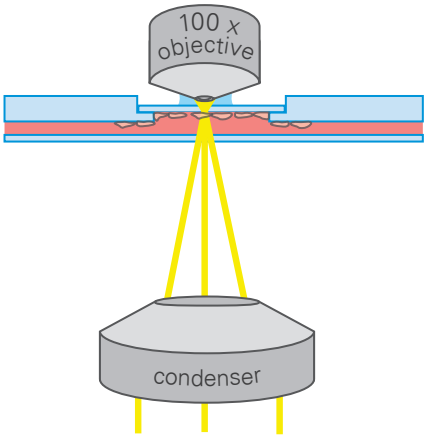
Technical features

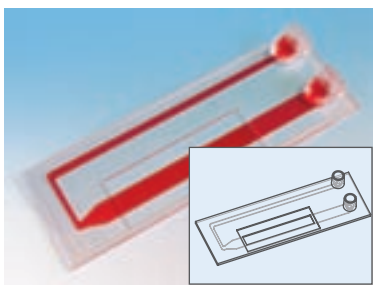
- window size of 30.7 mm x 5 mm, accessible with upright or inverted microscopes
- inner height of observation volume is 0.7 mm
- easy connection to tubes and pumps via female Luer adapters
- reservoir positions optimized for access with upright objective

Principle A
accessible with inverted microscopes



Principle B
accessible with upright microscopes





catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	80226
hydrophobic, uncoated / 15 pcs.	80221

technical details:

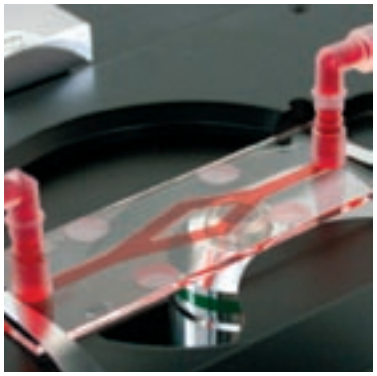
adapters	female Luer
recommended volume / reservoir	60 µl
number of channels	1
total volume	250 µl
volume of observation window	107 µl
height of observation window	0.7 mm
length of observation window	30.7 mm
width of channels	5.0 mm
growth area of observation field	1.53 cm²

µ-Slide y-shaped

Flow through µ-Slide for bifurcation studies and simulation of blood vessels

Applications

- 30° and 45° angles to simulate the bifurcation of a vessel
- designed for arteriosclerosis research
- ideal for cell-cell interaction studies and cell-drug interaction screenings under flow conditions

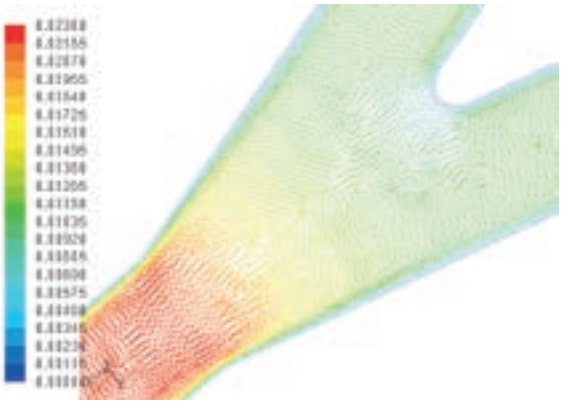


Technical features

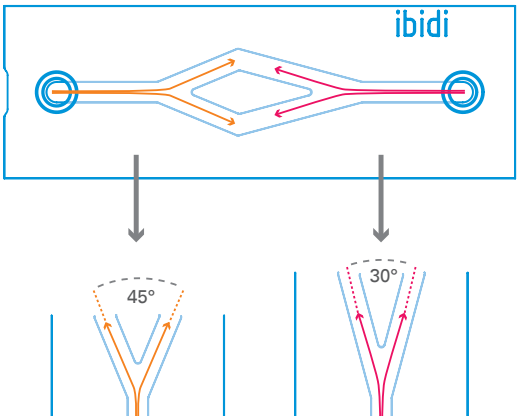
- ibiTreat surface ⇨ excellent for endothelial cell growth
- easy connection to exisiting tubes and pumps via female Luer adapter
- available as flow kit (see page 82)
- defined shear stress and shear rate analysis+

+ for detailed calculations please check www.ibidi.com

Non-uniform, laminar shear stress



Two flow directions possible



Please visit www.ibidi.com for more detailed information in Application Note 18

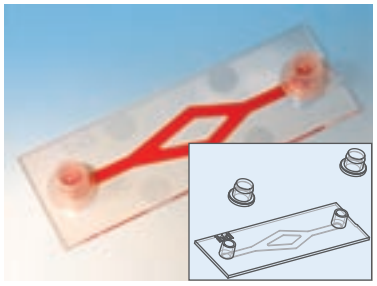
technical details:

adapters	female Luer
recommended volume per reservoir	60 µl
volume of the channel	110 µl
height of the channel	0.4 mm
growth area	2.8 cm²
bottom matches coverslip	No. 1.5

catalog numbers:

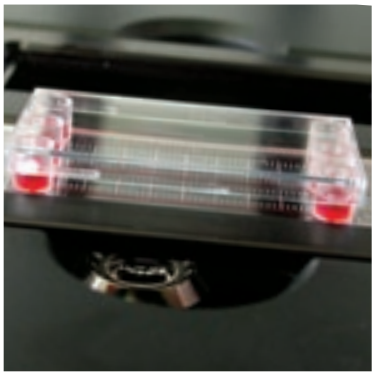
ibiTreat, tissue culture treated / 15 pcs.	80126
coated (Collagen IV) / 15 pcs.	80122
coated (Fibronectin)* / 15 pcs.	80123
coated (Poly-L-Lysine) / 15 pcs.	80124
coated (Poly-D-Lysine)* / 15 pcs.	80125
hydrophobic, uncoated / 15 pcs.	80121

** available on request only*



µ-Slide III 0.1

3 assays on one µ-Slide for perfusion with rare samples such as cells or blood



Applications

- mouse blood experiments
- rolling and adhesion assay
- long term cultures of cells under perfusion

Technical features

- channel cross section of 0.1 mm (height) x 1 mm (width)
- small sample amounts
- 1 mm scale for sample location
- low dead volume
- 3 parallel assays on one slide
- also available as flow kit, see page 83

dyne/cm²	µl/min
1	9.37
10	93.69
100	936.94

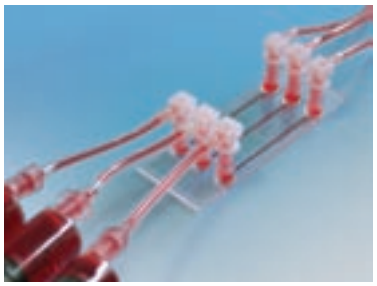
Low flowrate for physiological shear stress

Example experiment and reagent consumption

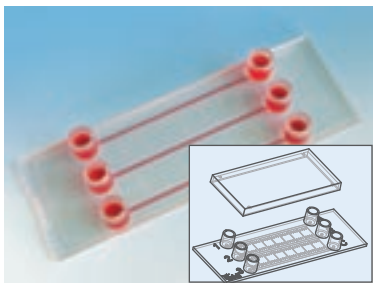
468 µl sample consumption only, in a one-way setup for following experimental parameters:

- shear stress 10 dyne/cm²
- duration 5 min = 300 sec
- viscosity 0.01 (dyne sec)/cm²

2 ml minimal sample volume for circular flow setup using the ibidi perfusion system (see pages 96-99)



Also available as flow kit see page 83



catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	80336
coated (Collagen IV) / 15 pcs.	80332
coated (Fibronectin)* / 15 pcs.	80333
coated (Poly-L-Lysine) / 15 pcs.	80334
coated (Poly-D-Lysine)* / 15 pcs.	80335
hydrophobic, uncoated / 15 pcs.	80331

** available on request only*

technical details:

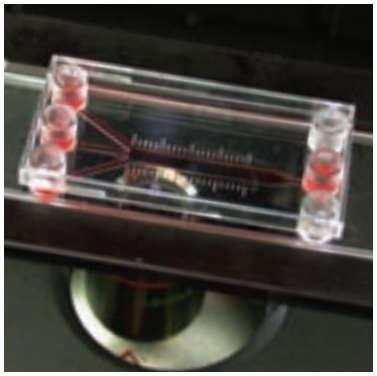
adapters	female Luer
recommended volume per resservoir	60 µl
number of channels	3
volume of each channel	4.5 µl
height of channels	0.1 mm
length of channels	45 mm
width of channels	1 mm
growth area per channel	0.43 cm²
distance of scale bars	1 mm

µ-Slide III 3in1

µ-Slide that merges 3 separated liquids into one channel in a laminar flow

Applications

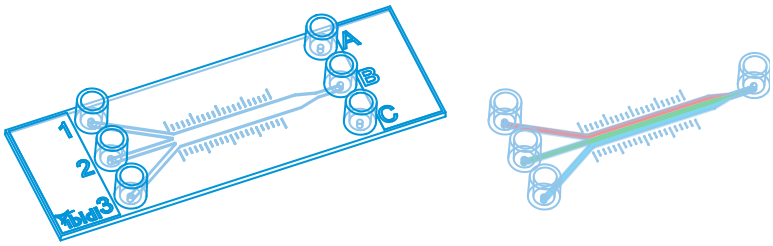
- fluidic assays with up to three different liquids
- cell sorting with laser traps
- fluidic focussing of inner lane

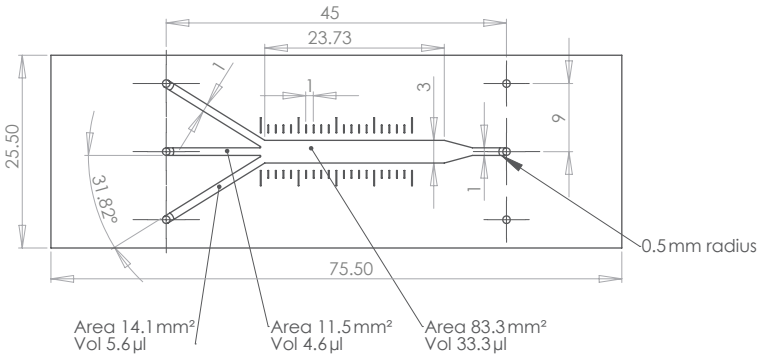


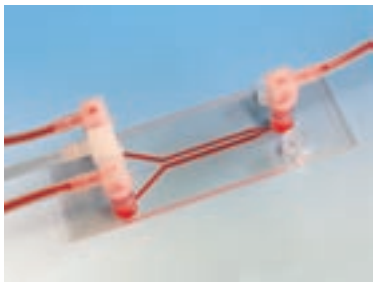
Technical features

- 3 channels end in 1
- luer connection
- low dead volume
- millimeter scale bar
- also available as flow kit, see page 83

Flow setup with three different liquids







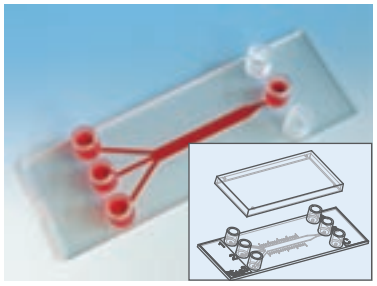
technical details:

adapters	female Luer
recommended volume per resservoir	60 µl
number of channels	3 in 1
total channel volume	60 µl
height of all channels	0.4 mm
width of channels thin/thick	1/3 mm
total growth area	1.23 cm²
distance of scale bars	1 mm

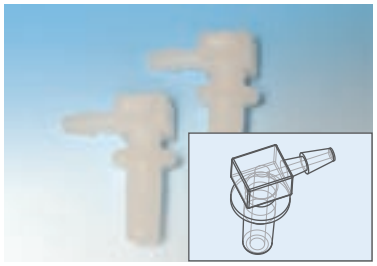
catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	80316
coated (Collagen IV) / 15 pcs.	80312
coated (Fibronectin)* / 15 pcs.	80313
coated (Poly-L-Lysine) / 15 pcs.	80314
coated (Poly-D-Lysine)* / 15 pcs.	80315
hydrophobic, uncoated / 15 pcs.	80311

** available on request only*



Elbow Luer connector



- allows connection to a female Luer adapter
- compatible design for setup within the ibidi heating system
- compatible with cell culture
- low height for optimized handling on microscopes

catalog number:

PP, sterile / 50 pcs.	10802

technical details:

male Luer adapter

suitable to	female Luer adapters
ID	2 mm

single barb

suitable to	flexible tubes, ID 0.8-1.6 mm
ID	0.8 mm

general:

total height	18 mm
temperature stability	up to 125 °C (autoclavable)
material	polypropylene
color	white
pressure max.	500 mbar

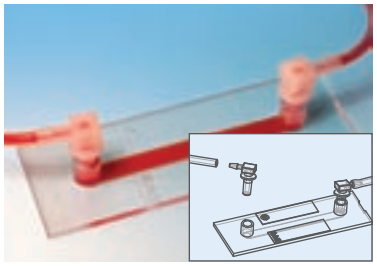
Flow kits in general

- technical data of the µ-Slides are given on the individual slide pages
- tubes and connectors are autoclavable
- easy connection to existing equipment (pumps, syringes, other tubes)

Packing list

- flow kits come with 1 µ-Slide and the required amount of tubes and connectors; e.g. for µ-Slide I Luer 2 connectors, 2 tubes (one in; one out) or for µ-Slide VI 12 connectors, 12 tubes (6 in and 6 out)
- tubes have a length of 20 cm each; ID 1.6 mm, OD 3.2 mm

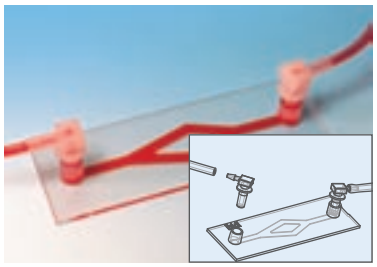
µ-Slide I Luer flow kit



- large microscopy area
- defined shear stress and shear rate analysis+
- for technical details of the µ-Slide I Luer see page 73

catalog numbers: µ-Slide I	0.1 Luer	0.2 Luer	0.4 Luer	0.6 Luer	0.8 Luer
ibiTreat, tissue culture treated, sterile	80046	80066	80076	80086	80096
coated (Collagen IV), sterile	80042	80062	80072	80082	80092
hydrophobic, uncoated, sterile	80041	80061	80071	80081	80091

µ-Slide y-shaped flow kit



- tests of active substances on cell lines
- vessel branching simulation for arteriosclerosis research
- defined shear stress and shear rate analysis+

catalog numbers:

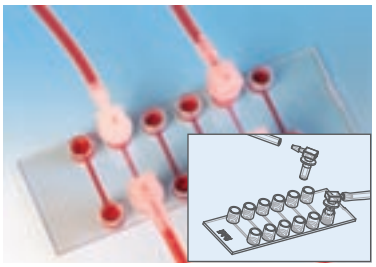
ibiTreat, tissue culture treated, sterile	80146
coated (Collagen IV), sterile	80144
hydrophobic, uncoated, sterile	80145

- for technical details of the µ-Slide y-shaped see page 79

+ for detailed calculations check www.ibidi.com

µ-Slide VI 0.1 flow kit

- minimal amount of reagents and cells needed
- large shear stress / flow rate ratio
- especially good for blood samples or rare cells
- defined shear stress and shear rate analysis+
- for technical details of the µ-Slide VI 0.1 see page 74

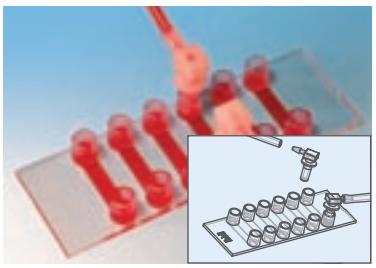


catalog numbers:

ibiTreat, tissue culture treated, sterile	80686
coated (Collagen IV), sterile	80682
hydrophobic, uncoated, sterile	80681

µ-Slide VI 0.4 flow kit

- suitable for toxicology and drug screening on cell lines
- six parallel flow assays
- especially for blood samples or rare cells
- defined shear stress and shear rate analysis+
- for technical details of the µ-Slide VI 0.4 see page 75

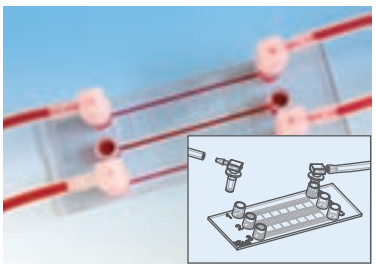


catalog numbers:

ibiTreat, tissue culture treated, sterile	80646
coated (Collagen IV), sterile	80642
hydrophobic, uncoated, sterile	80641

µ-Slide III 0.1 flow kit

- low amount of reagents and cells needed
- large shear stress / flow rate ratio
- defined shear stress and shear rate analysis+
- for technical details of the µ-Slide III 0.1 see page 80

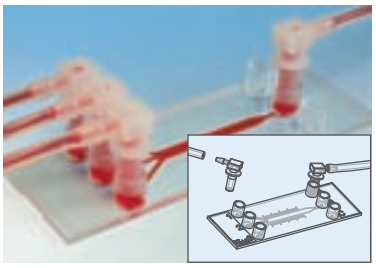


catalog numbers:

ibiTreat, tissue culture treated, sterile	81336
coated (Collagen IV), sterile	81332
hydrophobic, uncoated, sterile	81331

µ-Slide III 3in1 flow kit

- merges 3 liquids into 1 channel
- low amount of reagents and cells needed
- defined shear stress and shear rate analysis+
- for technical details of the µ-Slide III 3in1 see page 81



catalog numbers:

ibiTreat, tissue culture treated, sterile	81316
coated (Collagen IV), sterile	81312
hydrophobic, uncoated, sterile	81311

+ *for detailed calculations check www.ibidi.com*

µ-Slide VI – flat

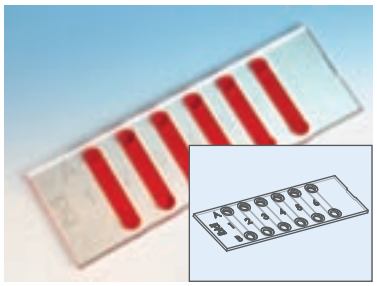
µ-Slide with 6 parallel channels for short term assays

Applications

- made for cell based short-time assays
- parallel screening of antibodies
- immunofluorescence assays using expensive or rare reagents

Technical features

- 30 µl test volume for low reagent amounts
- compatible with multi-channel pipettes
- no lid for long term cultivation ⇨ no sterile handling in long term assays!



catalog numbers:

ibiTreat, tissue culture treated / 15 pcs.	80626
coated (Collagen IV) / 15 pcs.	80622
coated (Fibronectin)* / 15 pcs.	80623
coated (Poly-L-Lysine) / 15 pcs.	80624
coated (Poly-D-Lysine)* / 15 pcs.	80625
hydrophobic, uncoated / 15 pcs.	80621

** available on request only*

technical details:

aperture Ø	4 mm
number of channels	6
volume of each channel	30 µl
height of channels	0.4 mm
length of channels	17 mm
width of channels	3.8 mm
growth area per channel	0.6 cm^2
bottom matches coverslip	No. 1.5

µ-Slide V

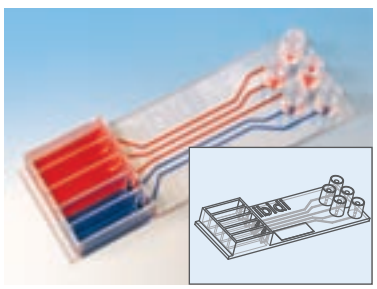
Protein assay µ-Slide for parallel solid phase immunoassays with extremely low sample amounts

Applications

- protein recognition and binding studies
- determination of binding constants based on fluorescence imaging

Technical features

- 10 µl channel volume ⇨ saves reagents
- adapters for all different pipette types



catalog number:

hydrophobic, uncoated / 15 pcs.	80501

technical details:

number of channels	5
volume of each channel	7.5 – 10 µl
height of channels	0.3 mm
length of channels	ca. 50 mm
width of channels	0.5 mm
bottom matches coverslip	No. 1.5

Custom specific flow slides and channels

develop your own geometry!

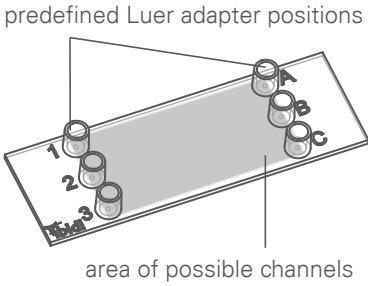
µ-Slides with custom-specific channel geometry and height

Applications

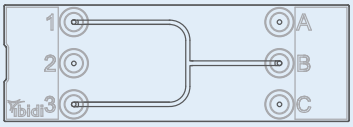
- create channels with your special characteristics
- microfluidic workbench
- cell culture flow applications

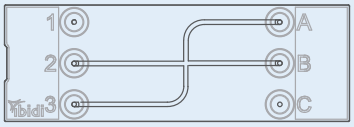
Technical features

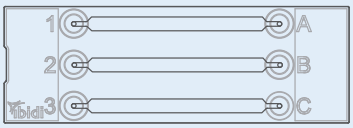
- format and material based on µ-Slides
- channel height and widths available in different dimensions
- 2 x 3 Luer adapters in fixed positions



Several possible variations:

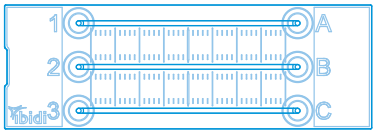

branching channels

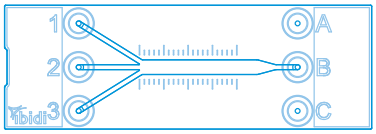

cross channels

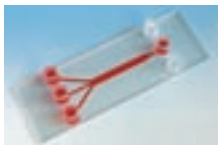

straight channels


meander channels

Developed formats:


µ-Slide III 0.1


µ-Slide III 3in1



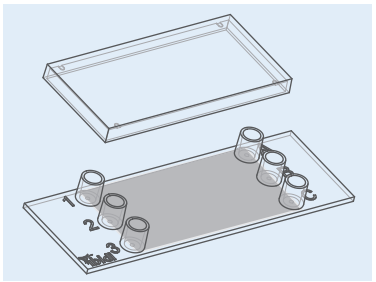
If you are interested in a custom specific solution please contact us directly at customer.solutions@ibidi.com.

technical details:

adapters	female Luer
recommended volume per resservoir	60 µl
height of channels	0.05 - 0.6 mm
width of channels	0.1 - 10 mm

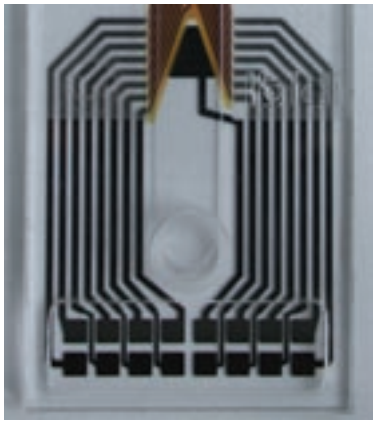
catalog numbers:

Please contact us for further details



Custom specific µ-Slide I Luer electrode

For microscopic and electric analysis of cells simultaneously



Applications

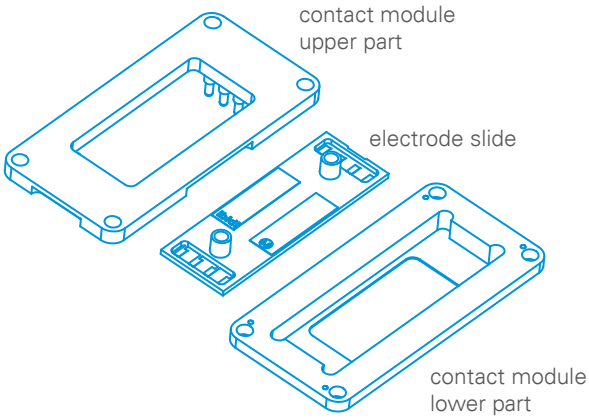
- applying and measuring defined voltage (electric fields)
- electrical stimulation of cells (e.g. heart muscle cells)
- electrotaxis (directed cell migration in an electric field)
- polarization of cells by electric fields
- apoptosis and necrosis assays by measuring resistance changes over the channel

Technical features

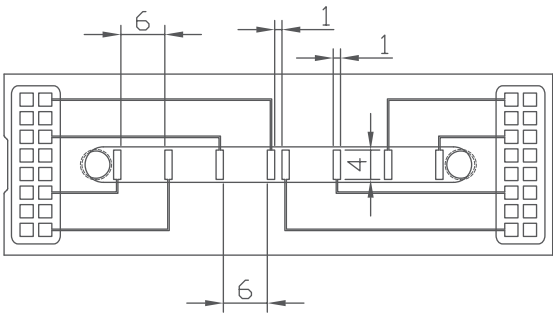
- resistance approx. 300 Ohm
- electrodes on glass support #1.5
- electrodes based on Ti-TiN layer
- available with different channel heights, ranging from 0.1 mm to 0.8 mm
- contact module with 8 – 32 ports
- contact module with integrated amplifier available on request
- insulated tracks available on request
- custom-specific electrode arrangements on request

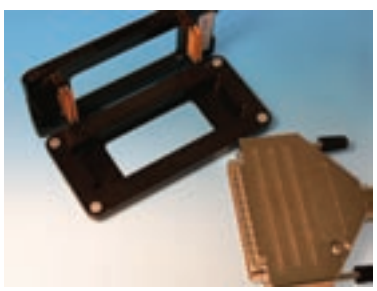
For ECIS analysis please visit pages 104 - 105

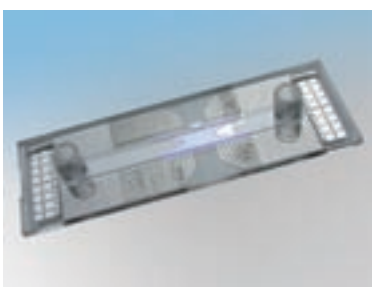
Contact module compatible with ibidi heating system



Electrode layout







catalog numbers:

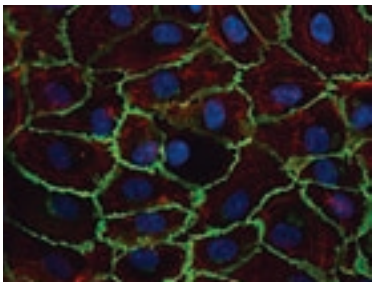
custom specific µ-Slide I Luer electrode / 15 pcs.	82000
contact module / 1 pc.	10939

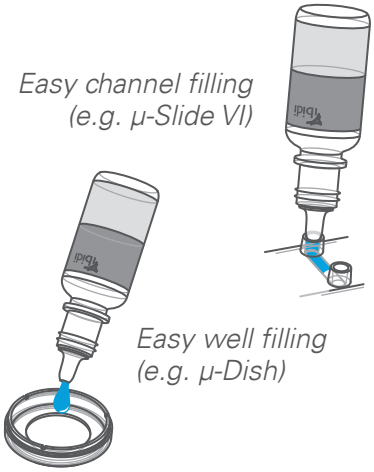
In cooperation with NMI, Natural and Medical Sciences Institute at the University of Tuebingen.

ibidi Mounting Medium

Mounting medium optimized for fluorescence microscopy

- especially designed for all ibidi µ-Slides, µ-Dishes, µ-Plates
- dropper bottle for exact dispensing into channels or open wells
- contains antioxidant agent which preserves fluorochromes
- no autofluorescence ⇨ bright fluorescence signals
- optimal pH for fluorescence signals
- non-drying and volume stabile
- non-hardening
- ready-to-use





catalog numbers:

ibidi Mounting Medium 15 ml	50001

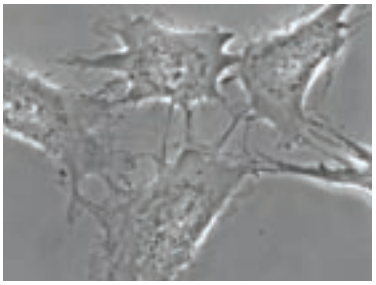
technical details:

appearance	optically clear
pH	8.5 - 9.0
storage of product	dark, room temperature
storage of mounted samples	dark, 4° C

ibidi Freezing Medium

Serum-free freezing of cells

- no preliminary freezing required
- direct freezing at -80°C or even in liquid nitrogen
- no sequential freezing required
- extremely high recovery rates
- packaging sizes of 10, 5 x 20 and 120 ml
- very competitive pricing



technical details:

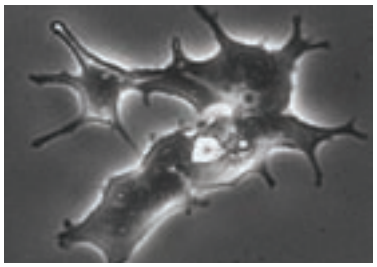
appearance	optically clear
storage of product	dark, 4° C

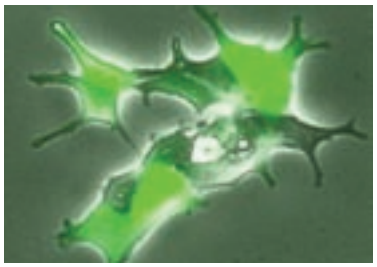
catalog numbers:

ibidi Freezing Medium 10 ml	80021
ibidi Freezing Medium 5 x 20 ml	80022
ibidi Freezing Medium 120 ml	80023

µ-Transfection Kit VI

For transfection of mammalian cells with METAFECTENE® µ in µ-Slide VI 0.4





Applications

- cell culture, transfection, and microscopy – all-in-one slide
- easy protocol – no optimization required
- fast results – two experiments per week

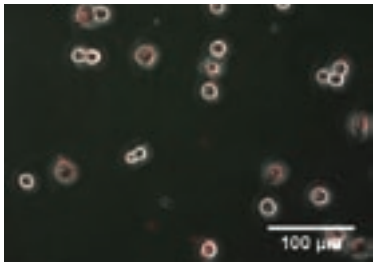
Technical features

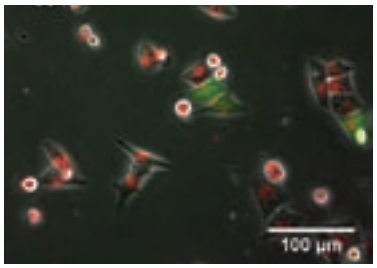
- kit contains METAFECTENE® µ and µ-Slide VI 0.4
- 90 transfection experiments per kit
- ideal optical properties for fluorescence microscopy

METAFECTENE® µ (Biontex Laboratories GmbH) was developed specifically for transfection of mammalian cells using ibidi µ-Slides VI 0.4. The reagent combines low toxicity with outstanding transfection results and simple, rapid protocol.

µ-Transfection Kit VI FluoR

For transfection of mammalian cells with Rhodamine labeled METAFECTENE® µ in µ-Slide VI 0.4





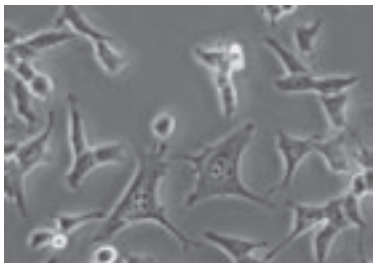
- features similar to µ-Transfection Kit VI but with a fluorescent labeled reagent
- ideal optical properties for fluorescence microscopy
- compatible with immersion oil and fixatives

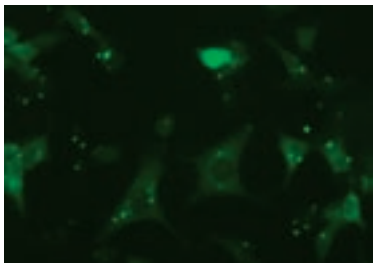
METAFECTENE® µ FluoR (Biontex Laboratories GmbH) is a fluorescence-labeled transfection reagent designed specifically for transfection of mammalian cells using ibidi µ-Slides VI 0.4. The Rhodamine labeled compound enables the transfection process to be visualized via tracing of the lipoplex.

catalog numbers:		technical details/content:	
µ-Transfection Kit VI	60601	µ-Slide (ibidi)	µ-Slide VI 0.4, ibiTreat (15x)
		Reagent (Biontex)	METAFECTENE® µ (100 µl)
		PBS (Biontex)	1x PBS (10 ml)
µ-Transfection Kit VI FluoR	60602	µ-Slide (ibidi)	µ-Slide VI 0.4, ibiTreat (15x)
		Reagent (Biontex)	METAFECTENE® µ FluoR (100 µl) (Rhodamine labeled, Exc_{max} 557 nm, Emi_{max} 571 nm)
		PBS (Biontex)	1x PBS (10 ml)

µ-Proteofection Kit VI

For direct insertion of antibodies/proteins into the cytosol of living cells





Applications

- direct transfer of antibodies/proteins through the membrane
- protein localization through fluorescent antibodies
- intracellular competitive inhibition
- replacing proteins and protein studies in knock down organisms

Technical features

- PROTEOfectene® optimized for protein insertion
- PROTEOfectene® AB optimized for antibody insertion
- kits contains PROTEOfectene® (AB), appropriate positive control reagents and µ-Slides VI 0.4
- fast and simple protocol
- 90 experiments per kit

The PROTEOfectene® reagents (Biontex Laboratories GmbH) were developed to enable the direct uptake of proteins and antibodies through the lipid membrane of cells by endocytosis. So that shuttled proteins reach the cytosol and can act directly in signal transduction. If labeled the fate of the AB/protein can be followed directly via microscopy.

For more details on the mechanism please check page 45.

technical details/content:

µ-Slide (ibidi)	µ-Slide VI 0.4, ibiTreat (15x)
Reagent (Biontex)	PROTEOfectene® (250 µl)
Positive control (Biontex)	R-Phycoerythrin, 100 µg/ml in PBS (100 µl)
µ-Slide (ibidi)	µ-Slide VI 0.4, ibiTreat (15x)
Reagent (Biontex)	PROTEOfectene® AB (250 µl)
Positive control (Biontex)	FITC-IgG, 100 µg/ml in PBS (100 µl)

catalog numbers:

µ-Proteofection Kit VI	60641
µ-Proteofection Kit VI AB	60642

Immersion oil

Immersion oil fully compatible with all µ-Slides, µ-Dishes and µ-Plates

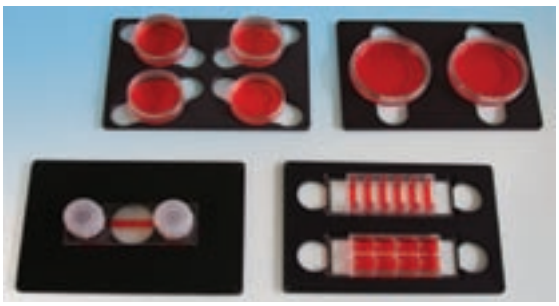
- compatible with all ibidi products
- excellent imaging quality
- packaging sizes of ca. 30 ml (1 fl oz), ca. 120 ml (4 fl oz)
- refractive index = 1.515
- ultra-low fluorescence background

catalog number:

Immersion oil ca. 30 ml (1 fl oz)	50101
Immersion oil ca. 120 ml (4 fl oz)	50102

Microscopy racks without lid

Various microscopy racks



- holder for 2 µ-Slides or 4 µ-Dishes (35 mm)
- outer dimension of a multi-well plate
- compatible with all microscopes
- no additional stabilization via a lid

catalog numbers:

4 x µ-Dish 35 mm	73402
2 µ-Slides	73401
general	73409
2 x µ-Dish 50 mm	73403

35 mm µ-Dish microscopy rack with magnetic fixation

ibidi microscopy rack for perfect µ-Dish stabilization

- holder for up to 6 µ-Dishes (35 mm high or low)
- compatible with all microscopes
- outer dimension of a multi-well plate
- additional stabilization via a lid fixed with magnetic positioning

catalog numbers:

ibidi µ-Dish microscopy rack, base	80035
magnetic lid for µ-Dish 35 mm, low / 1 pc.	80036
magnetic lid for µ-Dish 35 mm, high / 1 pc.	80037

µ-Slide microscopy rack with magnetic fixation

ibidi microscopy rack for perfect µ-Slide stabilization

- holder for up to 4 µ-Slides
- compatible with all microscopes
- outer dimension of a multi-well plate
- additional stabilization via a lid fixed with magnetic positioning

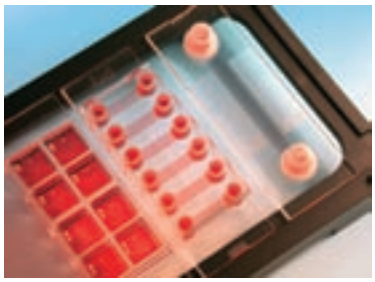
catalog numbers:

ibidi µ-Slide microscopy rack / 1 pc.	80030
magnetic lid for µ-Slides / 4 pc.	80031

µ-Slide rack

How to handle your slides in an incubator

- prevents scratching the µ-Slide bottom
- ideal storage after oil immersion microscopy
- allows easy gas exchange through the bottom of the µ-Slides
- stackable, made of aluminum
- convenient parallel handling for up to 8 µ-Slides
- rack height not directly compatible with microscopy
- easy slide transport from incubator to work bench



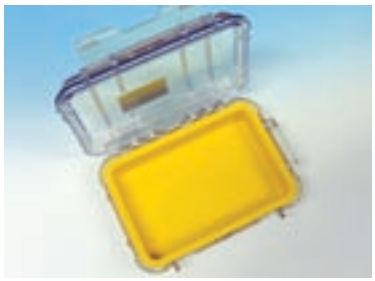
catalog number:

µ-Slide rack	80003

Olaf

Humidifying chamber for slides

- preserves humidity, temperature and gas concentration during incubation of cells (inside the incubator)
- combination with click rack possible
- inside dimensions fit multi-well format (127.5 mm x 85.5 mm)
- sterilization via alcohol / water mixture



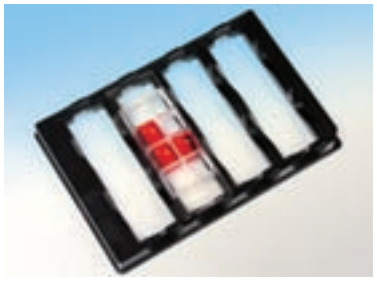
catalog numbers:

Olaf	80008
Olaf with click rack	80009

µ-Slide click rack

Where to place your µ-Slides

- parallel handling of up to 4 µ-Slides
- slides are fixed in their position on the rack
- outer dimensions of the rack equal to a multi-well plate (127.5 mm x 85.5 mm)
- fits into the humidity chamber Olaf
- rack height not directly compatible with microscopy
- not compatible with µ-Slide I Luer family



catalog number:

µ-Slide click rack	80007
µ-Slide click rack with Olaf	80009

Incubator

µ-Galaxy for µ-Slides

- small system with only 14 liter volume
- ideal for separated assays
- full CO_2 and heat control
- pre pressure of 0.35 bar only ⇨ saves gases
- robust in routine applications
- ideal for ECIS applications (see pages 104-105) and independent flow assays
- sealable hole for cables and tubes

Cultivating of cells normally happens at 37°C and 5 % CO_2. Therefore, incubators like the µ-Galaxy are used. That system addresses researchers' needs to manipulate the cells frequently which might happen with the ibidi µ-Slides or during ECIS measurements (see page 104). To prevent any contamination in the regular cell culture we recommend running those experiments separately.

The µ-Galaxy is characterized by a small volume. The incubator is a compact system that comes with a 14 l volume only. The system is 31 x 33 x 43 cm^3 and therefore requires very little lab space.

The backside of the µ-Galaxy comes with a sealable hole, so that cables or tubes can easily be directed outside. The incubator has two trays. So, it can hold racks or µ-Slides or ECIS slideholders easily. Due to its compact form, it requires only small amounts of CO_2.

catalog number:

incubator	10991

technical details:

volume	14 l
outer dimensions width x depth x heigth in cm	31 x 33 x 43
inner dimensions width x depth x heigth in cm	23 x 21 x 30
hole Ø	25 mm
pre pressure	0.35 bar

How to identify the ideal pump for your purpose

	syringe pump	peristaltic pump	ibidi pump system
pulsation	almost none (only initial pulse)	pulsation via drive shaft	almost none (only during valve activation)
circulating flow	possible but only with push and pull pumps	unlimited	unlimited
uni-directional flow	possible, but volume limited	possible, but volume limited	possible
mechanical stress for non-attached cells in the reservoir during pumping	almost none	strong	almost none
combination with microscopy	excellent	difficult due to pulsation	excellent
flow velocity	volume limited	unlimited	unlimited
parallelization	possible	possible	possible
set up in the incubator	difficult	difficult	easy
required volume	limiting factor	small	small
programmable	yes	yes	yes

Peristaltic pump

For long term parallel flow assays outside the incubator

- wide range of adjustable flow
- low pulsation rate
- allows parallel perfusion assays with µ-Slide VI flow kit
- changeable pump directions
- robust in routine lab work

The perfusion pump of the MCP series allows adjustment of a wide range of flows over the specimen. It comes with a relatively low pulsation rate and 8 individual flow channels. Therefore, it is perfectly suited for use with the µ-Slide VI flow kits.

It can easily be programmed, either for producing flow ramps or to adjust the flow direction. It is a very robust model in routine lab work. Delivery includes a CA-8 pump head.

catalog number:

Peristaltic pump with CA-8 pump head	10901
Tygon tube stoppers	10909

KD Scientific syringe pumps

KDS 100 combines precision with simplicity

- single syringe pump
- simple installation
- 10 µl to 60 ml syringes of all types
- precise flow delivery
- minimal shear of the cells in the syringe
- KDS 101 holds two syringes, 10 µl to 10 ml each

catalog numbers:

KDS 100	10940
KDS 101	10941

technical details:

syringes: max. No. and size:	KDS 100 / 101	one / two; 10 µl to 60 ml / 10 µl to 10 ml
min. flow rate:	KDS 100 / 101	0.1 / 0.001 µl/h
max. flow rate:	KDS 100 / 101	519 ml/h / 0.35 ml/min

KD Scientific syringe pumps and infusion pumps provide an accurate delivery of fluids for the laboratory environment. The series are divided in an economical line of uni-directional infusion pumps and a high end line with syringe pumps having infuse and withdraw capability. Minimum and maximum flow rates depend on the size of the syringe you are using.

Infusion pumps

KD Scientific infusion pumps are ideal for delivering accurate and precise amounts of fluids for a multitude of applications, including injection of calibrant into a mass spectrometer or reaction chamber, long term drug delivery to animals, and general infusion applications.

Infusion / withdrawal pumps

Infuse and withdraw capabilities provide maximum flexibility for varied applications. This feature permits applications such as automatic withdrawal of samples and unattended filling of syringes at very low flow rates.

Push / pull syringe pumps

These proven KDS pumps provide simultaneous infusion and withdrawal with opposing syringes on a single drive. The KDS 120 and KDS 260 are adaptations of the KDS 100 and KDS 210 respectively. Each one has been modified to hold an additional syringe, so that one syringe infuses, while the second one withdraws simultaneously.

KDS 101

KDS 250 to create up to 4 independent flows

- four syringes, 10 µl to 10 ml each
- perfect combination with µ-Slide III [3in1]
- minimal shear of cells
- separate clamping accommodates different sizes of syringes

technical details:

syringes: max. No. and size:	4, 10 µl to 10 ml each
min. flow rate (10 µl syringe):	0.001 µl/hr
max. flow rate (140 ml syringe):	1270 ml/hr

catalog numbers:

KDS 250	10951

KDS 270 for push/pull applications

- continuous flow system
- two syringes infuse/two withdraw at the same time
- multiple syringe pump
- simple installation
- small amounts of media required

technical details:

syringes: max. No. and size:	four; 10 µl to 30 ml each
min. flow rate (10 µl syringe):	0.001 µl/h
max. flow rate (30 ml syringe):	86 ml/min

catalog numbers:

KDS 270	10947

KDS 220 for applications with multiple syringes

- multiple syringe pump (up to 10 syringes in parallel)
- simple installation
- programmable version: KDS 220 P
- wide range of applications
- precise flow delivery

technical details:

syringes: max. No. and size:	ten; 10 µl to 10 ml each
min. flow rate (10 µl syringe):	0.001 µl/h
max. flow rate (10 ml syringe):	21 ml/min

catalog numbers:

KDS 220	10949

KDS 410 for high pressure applications

- extra force to deliver viscous fluids
- delivery of fluid to reactors in chemical applications
- to host steel syringes
- wide variety of syringes from 10 µl to 140 ml

technical details:

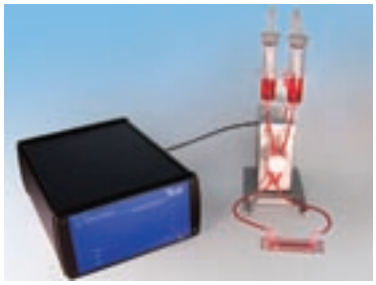
syringes: max. No. and size:	one, 10 µl to 140 ml
min. flow rate (10 µl syringe):	21.1 µl/min
max. flow rate (140 ml syringe):	1470 ml/min

catalog numbers:

KDS 410	10956

ibidi pump system

For long term uni-directional and/or oscillating flow assays



catalog numbers:

complete ibidi pump system	10902
ibidi air pressure pump	10905
fluidic unit	10903
notebook	10908

accessories for ibidi pump system

perfusion set, 15 cm, ID 0.8 mm, 10 ml (3 units)	10961
perfusion set, 15 cm, ID 1.6 mm, 10 ml (3 units)	10962
perfusion set, 50 cm, ID 0.8 mm, 10 ml (3 units)	10963
perfusion set, 50 cm, ID 1.6 mm, 10 ml (3 units)	10964
reservoir set, 10 ml, sterile (10 units)	10971
valve connection cable	10981

Applications

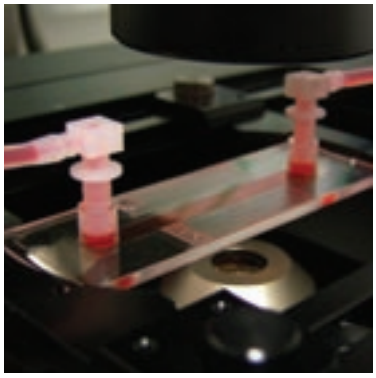
- cultivation of cells under flow conditions, inside an incubator in long term assays
- cell culture under defined shear stress
- enrichment of metabolic products as soluble factors
- long term flow experiments with a minimal amount of medium and supplement
- endothelial cell interaction with suspended cells under perfusion conditions

Cultivation under flow

Cultivation of endothelial cells under perfusion reflects their natural environment far better than doing so under static conditions. ibidi developed a perfusion system to address exactly that question. The system consists of two main components: a control and a fluidic unit. The major advantage of that "split" approach is that the closed flow setup can be assembled and after cultivation transferred to the microscope – without compromising the sterility of the system. The open architecture with the Luer connectors allows the use of any kind of flow devices.

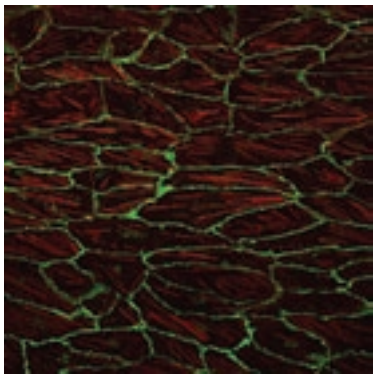
The ibidi perfusion system

The control unit is a computer controlled air pump. It generates pressure ranging from -100 to 100 mbar with +/- 1 mbar accuracy. Due to the fact that the flow is generated via air pressure, the mechanical stress on suspended cells in the reservoirs is minimized. The software is a convenient and flexible tool to control the pressure and in turn the shear rate. The fluidic



unit contains two reservoirs for the cell media, a slide holder and all required tubes for connecting reservoirs and µ-Slides. ibidi offers a range of perfusion sets with different diameters and lengths. Therefore these perfusion sets allow for varying the flow rates as well as placing the fluidic unit on the microscope stage. A set of valves generates an uni-directional flow.

The control unit is connected to the fluidic unit by a thin, low-voltage electric cable and a tube for transducing the air pressure which drives the flow. The air tube and the electric cable can be mounted into the incubator, either through a hole in the back wall, or alternatively through the front door seal.



For detailed information on cell based perfusion assays, please see page 30.

Technical features

- uni-directional, continuous flow
- oscillating flow possible
- fully compatible with ibidi heating stage
- small volume/circular system
- perfectly compatible with microscopy
- perfectly compatible with incubator conditions
- minimal mechanical stress on suspended cells
- up to four parallel fluidic units per air pump
- fully controllable via PC

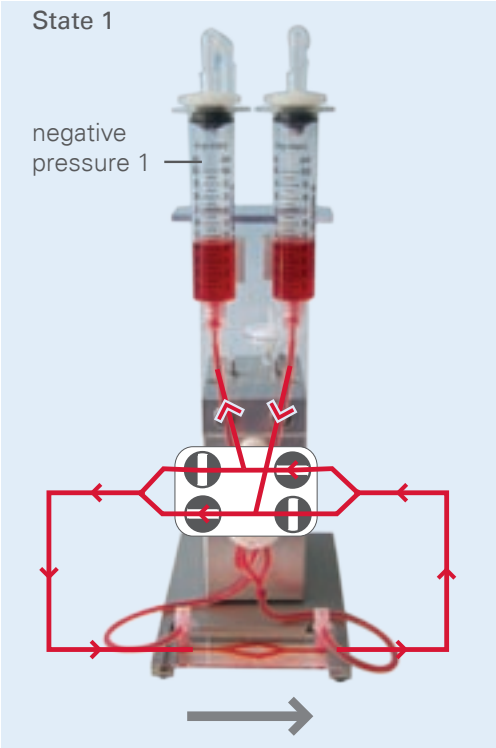
technical details:

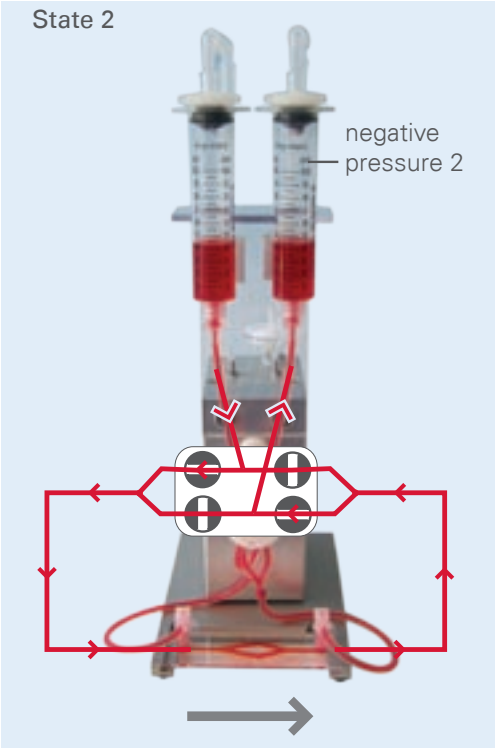
flow rate	0.5-50 ml / min
shear stress	0.5-85 dyne/cm²
working volume	3-15 ml
recommened working volume	10 ml
suitable for all µ-Slides with Luer adapters	

Experimental setup in an incubator

The system is a fully integrated solution. Hosting the fluidic unit in the incubator still allows for running perfusion assays directly at the microscope. A defined temperature and CO_2 concentration is assured as the fluidic reservoirs are inside the incubator at all times.

ibidi pump system

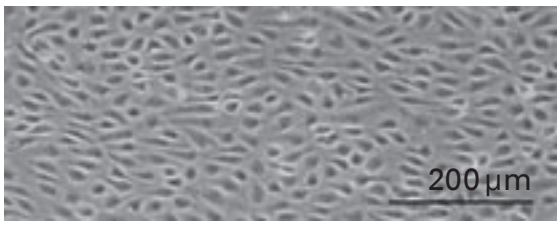




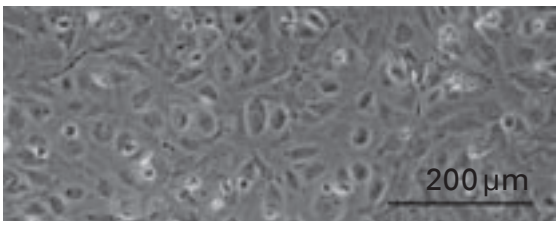
Principle of flow generation

The pressure generator works with a negative pressure. At state 1 the negative pressure is supplied at reservoir 1. Consequently, medium is sucked from reservoir 2 filling reservoir 1. As soon as reservoir 1 is filled, all valves switch and the liquid is now sucked from reservoir 1 – via the same way and therefore in the same direction – filling reservoir 2.

Flow is a parameter



HUVECs in µ-Slide I $^{0.2}$ Luer cultivated for seven days at a flow rate of 3.9 ml/min (20 dyn/cm²). The cells show a good orientation in the direction of the flow.



HUVECs in µ-Slide I $^{0.2}$ Luer cultivated for seven days under static conditions. The medium has been changed every day.

Advantages

- fluidic unit can be placed into the incubator while the control unit is outside the incubator
- fluidic unit and control unit can be separated without losing sterility; makes preparation and live observation on the microscope easy
- compatible with all incubators
- minimized mechanical stress on suspended cells such as monocytes
- uni-directional flow using small amounts of reagents
- easy handling with disposable flow chambers and Luer connectors

Flow generated with various µ-Slides and perfusion sets

Perfusion set 15 cm, ID 0.8 mm, blue

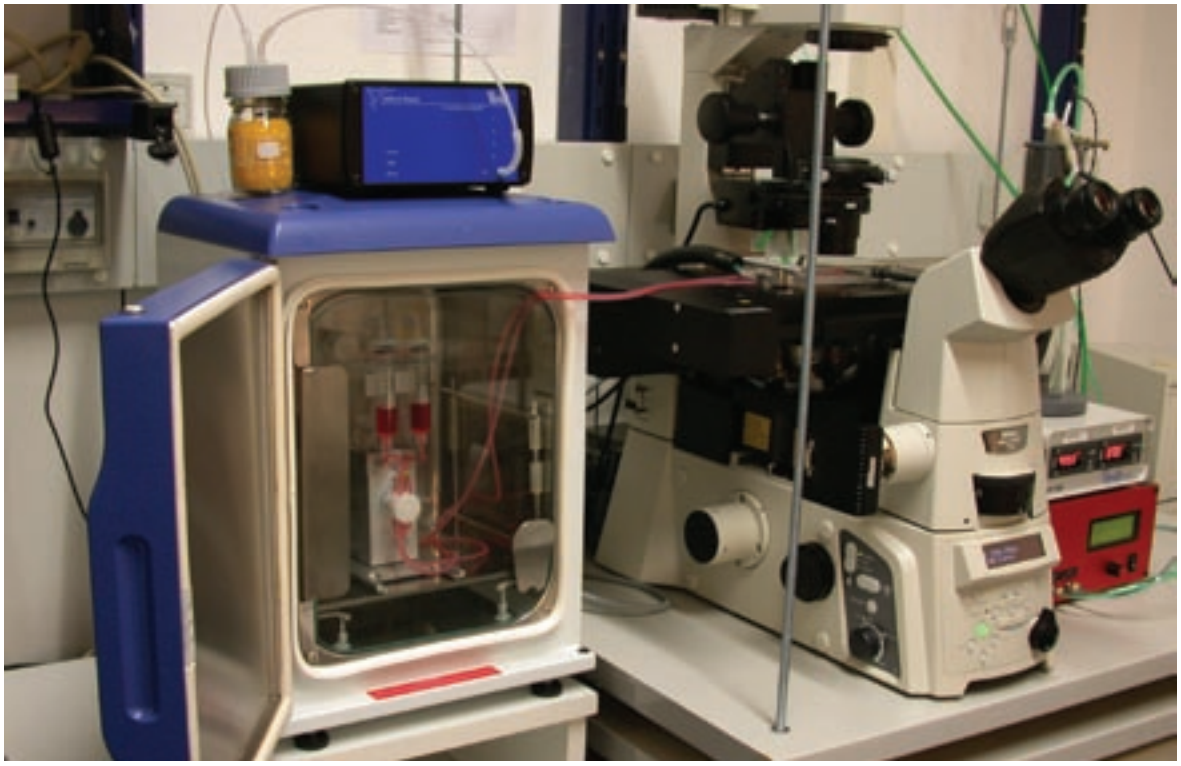
	Flowrate min [ml/min]	Flowrate max [ml/min]	Shear stress min [dyne/cm²]	Shear stress max [dyne/cm²]
µ-Slide I 0.2 Luer	0.5	8.5	2.6	43.6
µ-Slide I 0.4 Luer	0.7	10.2	0.9	13.4
µ-Slide I 0.6 Luer	0.7	10.9	0.4	6.6
µ-Slide I 0.8 Luer	0.8	12.1	0.3	4.2
µ-Slide VI $^{0.4}$	0.7	9.2	1.2	16.2
µ-Slide y-shaped	1.2	17.8	2.7	40.5

Perfusion set 15 cm, ID 1.6 mm, red

	Flowrate min [ml/min]	Flowrate max [ml/min]	Shear stress min [dyne/cm²]	Shear stress max [dyne/cm²]
µ-Slide I 0.2 Luer	0.9	14.4	4.6	73.9
µ-Slide I 0.4 Luer	2.1	26.5	2.8	34.9
µ-Slide I 0.6 Luer	2.4	30.0	1.4	18.0
µ-Slide I 0.8 Luer	2.6	30.8	0.9	10.7
µ-Slide VI $^{0.4}$	2.4	25.8	4.2	45.4
µ-Slide y-shaped	2.0	23.2	4.5	52.8

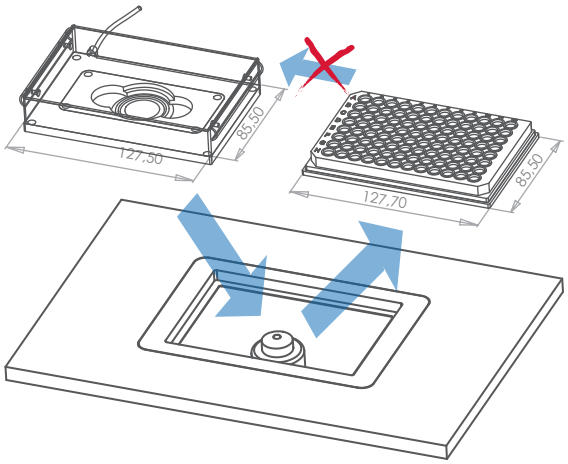
*We **highly recommend** perfusion sets ID 0.8 mm (10961, 10963) for flow rates from 0.1 - 11 ml/min and perfusion sets ID 1.6 mm (10962, 10964) for flow rates from 1.1 - 60 ml/min.*

Example of a perfusion set-up on a microscope



Heating stages and incubation units

Heating stages for live cell imaging



- universal heating system for all microscope platforms
- micro environment via heated plate and heated transparent lid
- heated transparent lid prevents condensation on µ-Slide lids
- with inserts for µ-Slides, regular slides, or 35 mm dishes
- controller for two separate heating systems
- optional with gas incubation unit

Please note that the outer dimensions of the heated stage have a multi-well format. Therefore, it cannot host multi-well plates.

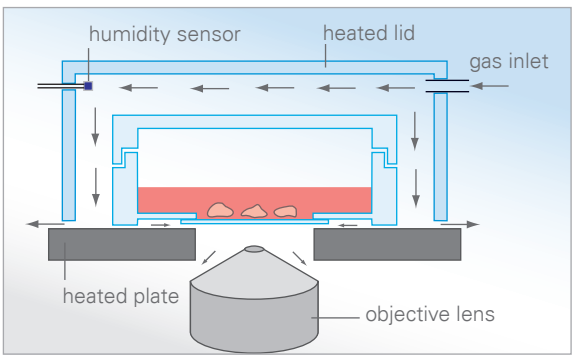
Imaging cells *in vitro* is one of the challenges in today's microscopy. The two critical parameters that need to be monitored closely are pH and temperature. To monitor and regulate changes in temperature, we developed a heating system that allows for easy temperature control in a very cost effective manner. The heating device can be used with any inverted microscopy system and can be adapted to any microscopy chamber or slide.

This heating stage is a two component system that consists of a heated plate and a heated transparent lid. The base for the heated plate has a multi-well format. In this way, the system can be placed into any regular multi-well frame, delivered with your microscope. So, we have generated a heating device that can be used on microscopes from different manufacturers. It fits into both, manual and automated stages. The heated plate hosts all types of microscopy chambers as µ-Slides and µ-Dishes as well as non ibidi formats.

The heating elements are arranged in such a way that a defined temperature gradient in vertical direction arises. Warming the heated lid to around 40 °C and the heated base plate only to around 37 °C prevents the formation of condensed water at the lid of your microscopy chamber.

technical details:

multi-well plate sized heating frame (dimensions in mm)	127.5 x 85.5
temperature stabiliy (HT 200 / HT 50)	+/- 0.5 °C
weigth with insert	330 g
cut-out area in heated base plate	82 x 40 mm
heigth of the system	26.5 mm



That in turn, prevents scattering of light or shadow formation and provides a better illumination of the specimen.

Controllers for the heating systems

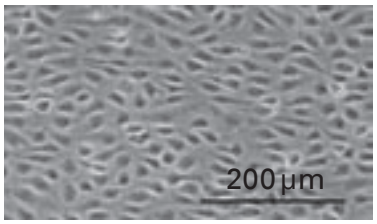
ibidi offers two different types of controllers to regulate the heat. The HT series comes with a digital heat control. This economic controller is optimized for reliability and is used in cell culture labs world wide. It is used in delicate applications such as live cell imaging and *in vitro* fertilization. This controller covers 90% of all applications.

In contrast, the ibidi T series heat controllers work with continuous current. Therefore, the T series leads to perfect focus stability and serves extremely high resolution applications at numerical apertures of NA >1.2. The optimization for extremely low focus drift makes the system especially suitable for techniques like TIRF and confocal microscopy. The continuous signal minimizes noise to an extremely low level. That makes these controllers suitable for electronic analysis of cells in patch clamp or impedance measurements.

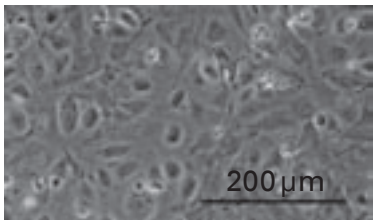
Gas mixers for the incubation unit

Additionally, the heated lid comes with a gas inlet. A variety of gas mixers upgrades the system to a complete stage top incubator.

I) active CO_2 mixer	mixes pressurized air and pure CO_2 to various mixtures (1 - 15 % CO_2)
II) active flexible gas mixer (CO_2, N_2, O_2, air)	for various gas mixtures; creates regular CO_2 and hypoxia conditions
III) passive system	for premixed gases only

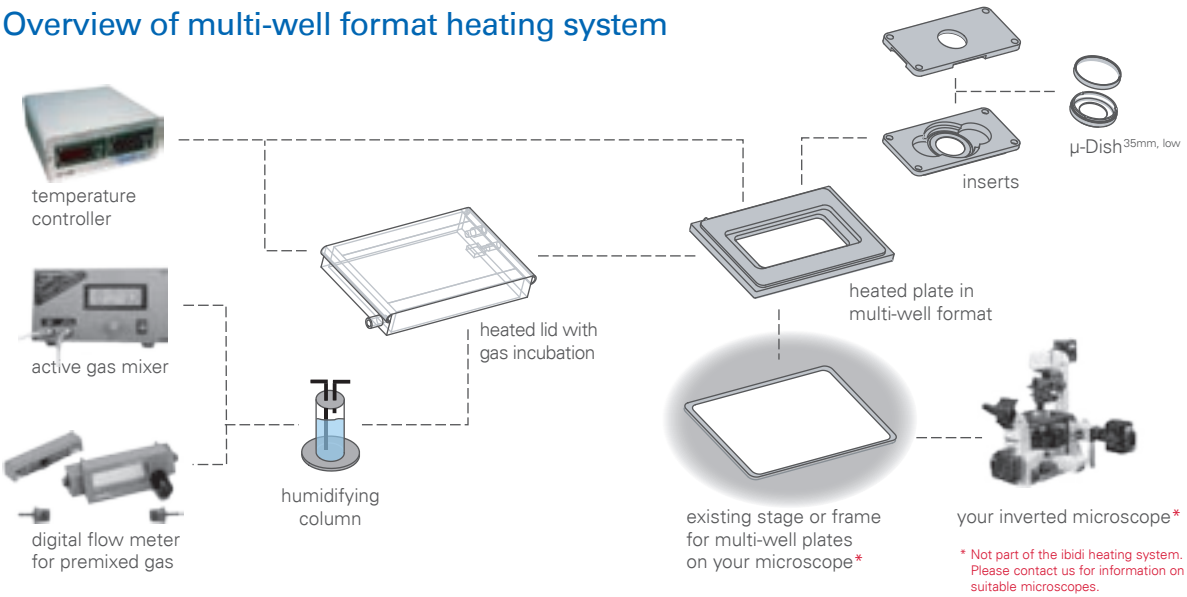


flow cultivation



static cultivation

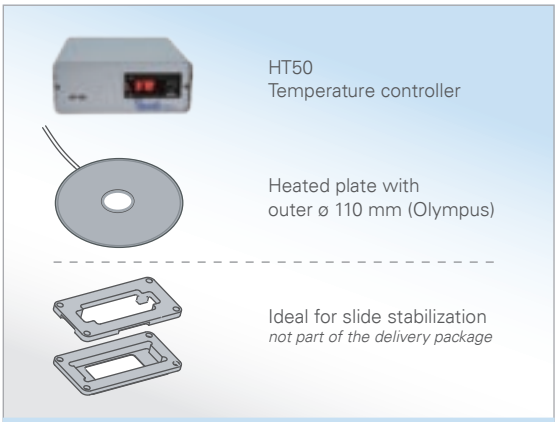
Overview of multi-well format heating system



* Not part of the ibidi heating system. Please contact us for information on suitable microscopes.

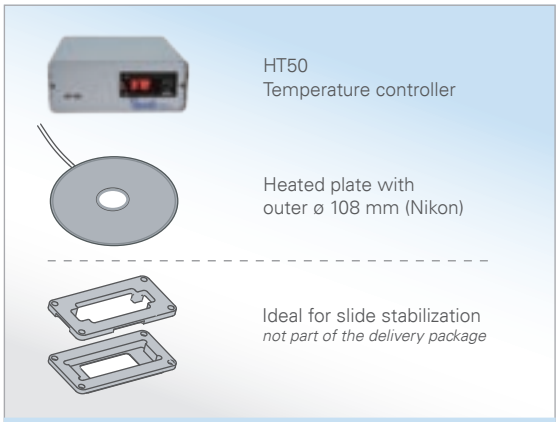
Heating systems

System 1



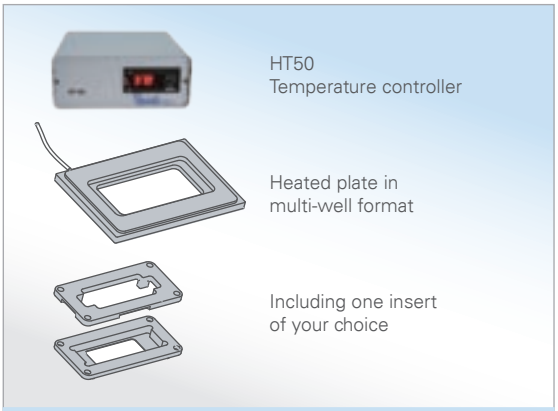
Heating system 1 10914

System 2



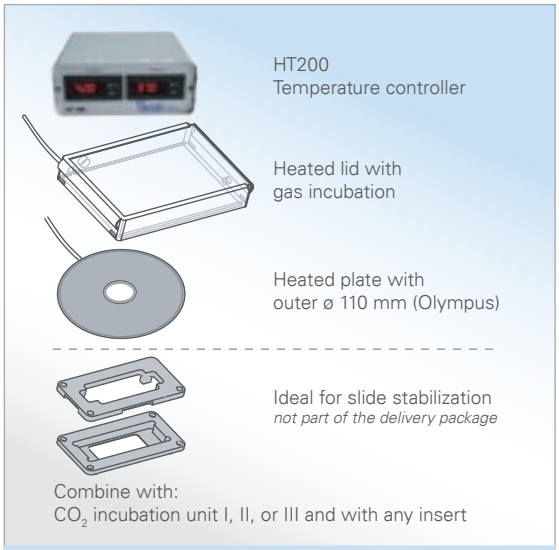
Heating system 2 10913

System 3



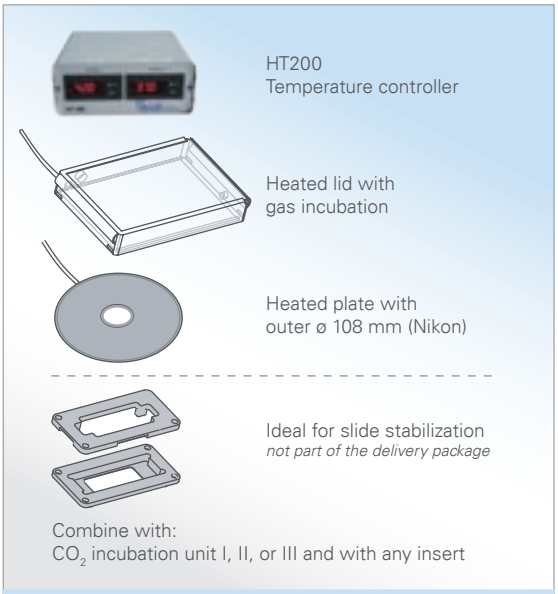
Heating system 3 10915

System 4



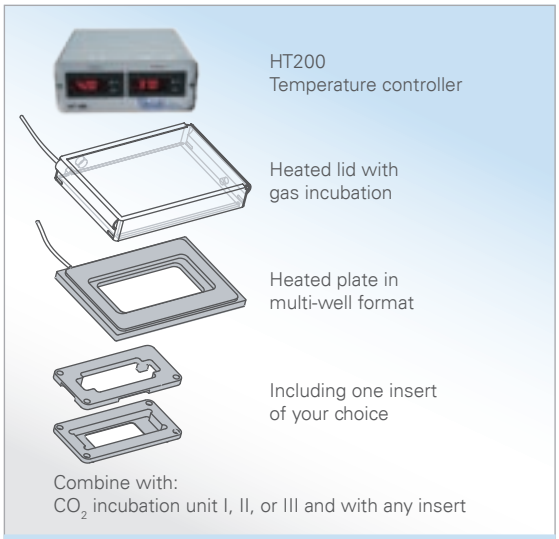
Heating system 4 10917

System 5



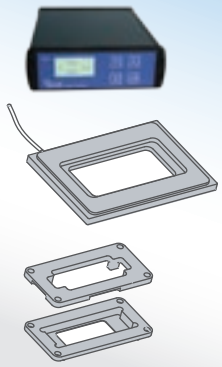
Heating system 5 10916

System 6



Heating system 6 10918

System 7



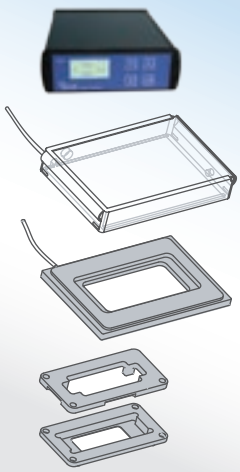
TC01
Temperature controller

Heated plate in
multi-well format

Including one insert
of your choice

Heating system 7 10919

System 8



TC02
Temperature controller

Heated lid with
gas incubation

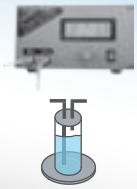
Heated plate in
multi-well format

Including one insert
of your choice

Heating system 8 10925

Gas incubation systems

Unit I
for CO_2 and pressurized air



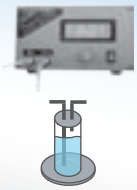
Active gas mixer
controls CO_2 levels

Humidifying column

Combine with: System 4, 5, 6, or 8

CO_2 gas incubation unit I / inlets for CO_2 and pressurized air 10920

Unit II
for various gases



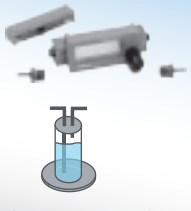
Active gas mixer
contols CO_2 and O_2 levels

Humidifying column

Combine with: System 4, 5, 6, or 8

CO_2 gas incubation unit II / inlets for various gases 10922

Unit III
passive system for premixed gas



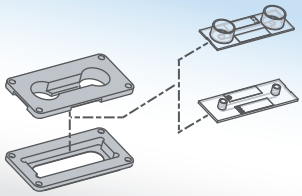
Digital flow meter
for premixed gas

Humidifying column

Combine with: System 4, 5, 6, or 8

CO_2 gas incubation unit III / passive system 10921

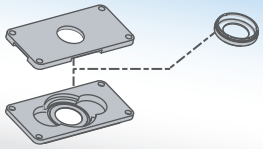
Heating inserts
and fitting µ-Slides / µ-Dishes



µ-Slide I

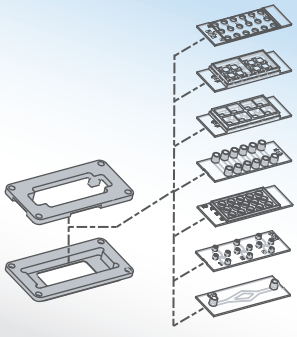
µ-Slide I Luer

Insert for µ-Slide I, µ-Slide I Luer 10930



µ-Dish^{35mm,low}

Insert for µ-Dish 35mm, low 10932



µ-Slide 18 well

µ-Slide 2x9 well

µ-Slide 8 well

µ-Slide VI

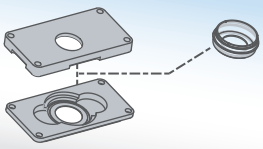
µ-Slide Angiogenesis

µ-Slide Chemotaxis

µ-Slide y-shaped

and all other ibidi µ-Slides
except µ-Slide I

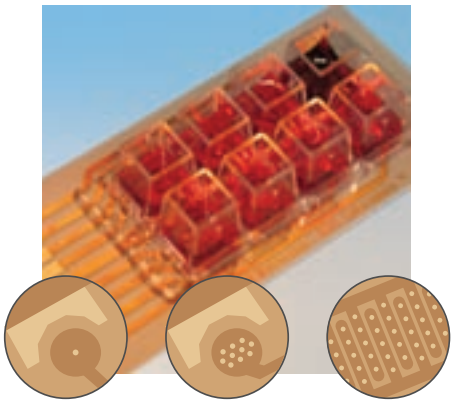
Insert for various µ-Slides 10933



µ-Dish^{35mm,high}

Insert for µ-Dish 35mm, high 10934

ECIS Technology



8W1E 8W10E 8W10E+

The Electric Cell-substrate Impedance Sensing (ECIS) is an automated method to monitor the cellular behaviour. The ECIS technology allows the observation of e.g. cell proliferation, cell attachment and spreading, motility and much more.

In the system, cells are grown upon small gold film electrodes carrying very weak AC signals. As cells attach and spread upon these electrodes, their insulating membranes block and constrain the current flow resulting in measured variations in the electrode impedance. Any changes in the environment that result in morphological changes of the cell alter the current paths and can be readily detected by the instrument. These changes may be due to the addition of compounds, changes in the physical environment like perfusion, transfections, viral infections and the addition of other cells. All measurements are made continuously, in real-time and without the need for any labels. As the minimal AC currents used in the measurement have no effect upon the cells; the method is totally non-invasive.

For more than 300 scientific publications using the ECIS technology please visit www.biophysics.com.

There are two major systems the ECIS Z and the ECIS Z-Theta. Both systems are available as 16 well systems or 96 well units.

The heart of the ECIS measurement is the electrode-containing array, where cells are cultured. These arrays come as 8 well slides and 96 well plates for higher throughput applications. Holders are available for two eight well slides or a single 96 well plate; both are hosted in an incubator, so cells are kept under ideal cultivation conditions – at all times.

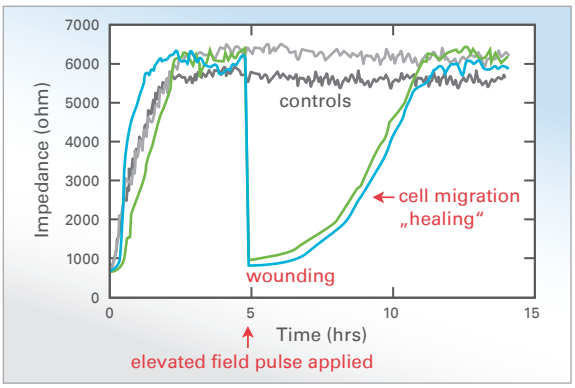
catalog numbers:

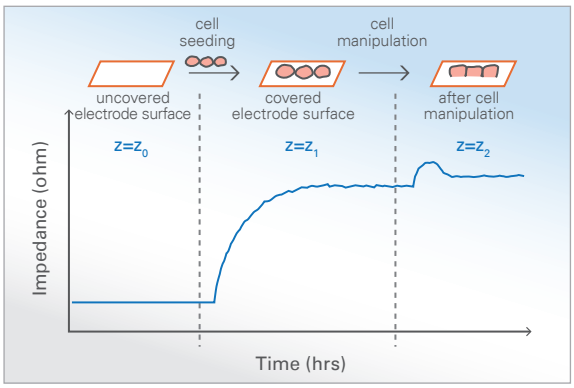
equipment

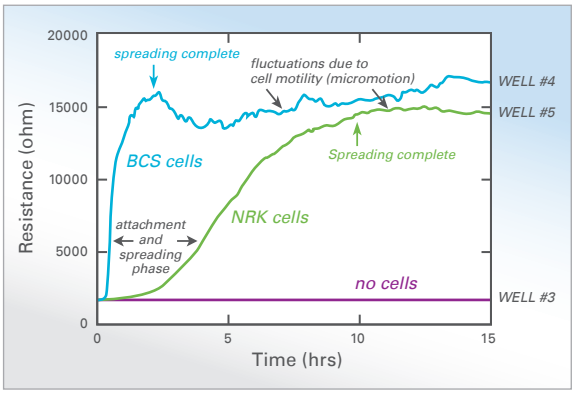
ECIS Model Z	71610
ECIS Model Z Theta	71611
16 well holder	71612
96 well holder	71614
Elevated field module (16 w)	71613
Elevated field module (96 w)	71615
Flow module	71001

impedance arrays

8W10E / 6 pcs.	70010
8W10E+ / 6 pcs.	70040
8W1E / 6 pcs.	70001
8W2x1E (Medusa) / 6 pcs.	70002
ECIS Flow Array / 6 pcs.	70101
8W1E (DD) / 6 pcs.	70003
96W1E / 5 pcs.	70096
96W10E / 5 pcs.	70095
8W1E (PCB) / 6 pcs.	70004
8W10E (PCB) / 6 pcs.	70011

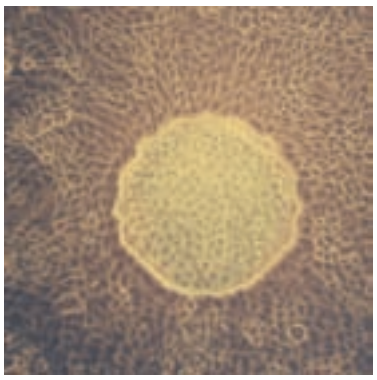


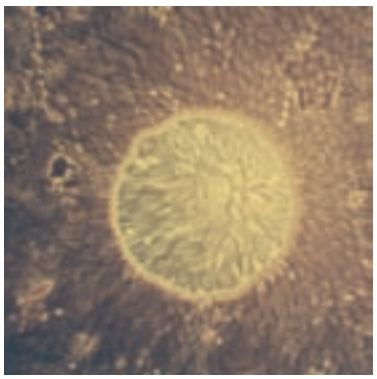




ECIS can be used for many cell-based assays including:

- cell proliferation and migration
- measurements of cell attachment and spreading on ECM proteins
- wound healing & electroporation
- toxicological screenings
- cytopathic effects of viral infections
- drug uptake studies
- measurement of endothelial layer permeability (under flow if desired)
- measurements of metastatic potential (cell invasion)
- signal transduction/GPCR screens

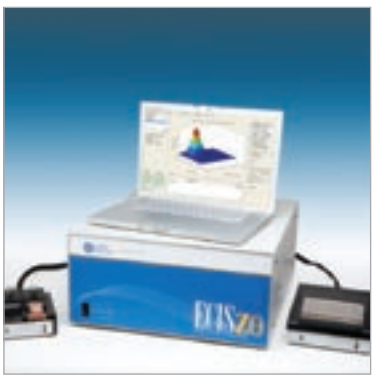


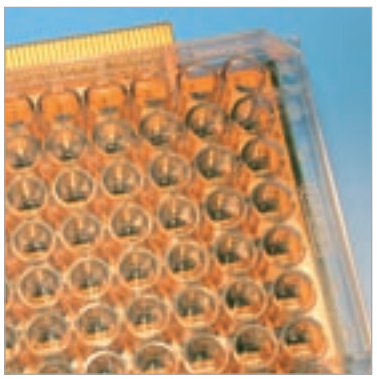


The Z-Theta system can also measure complex impedance at defined AC frequencies, allowing for modelling software to present:

- the barrier function of the cell layer
- the averaged cell membrane capacitance
- the distance of the cell from its substrate

For more detailed information please contact us.





The Extracellular Flux System

Determine cellular bioenergetics in a microplate

The XF Extracellular Flux Analyzer is a fully-integrated instrument that simultaneously measures the two major energy yielding pathways – aerobic respiration and glycolysis. The XF assays provide increased throughput in a drug discovery format. This fast and sensitive measurement of cellular bioenergetics allows to

- study integrated drug delivery for compound or complex experiments
- measure Oxygen Consumption Rate (OCR)
- determine Extracellular Acidification Rate (ECAR)
- measure in a label free and a time-resolved manner
- analyze in a microplate-based format (24 well / 96 well) with less variation
- reuse the cells after the non-destructive/non-invasive measurement

The Seahorse series comes with 3 versions. All systems determine the Oxygen Consumption Rate (OCR) and the Extracellular Acidification Rate (ECAR). The XF24/3 measures additionally CO_2 production rate (CDPR). For each well there are multiple injection ports so that complex assays or concentration studies can be performed easily.

catalog numbers:

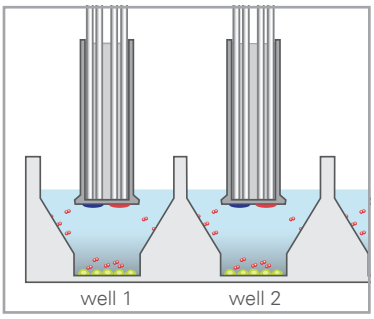
equipment

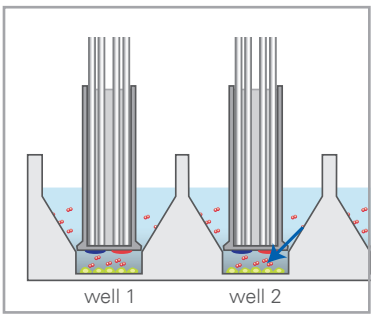
XF 24 Analyzer	75001
XF 24-3 Analyzer	75002
XF 96 Analyzer	75003
Prep station for XF 96	75004

disposables

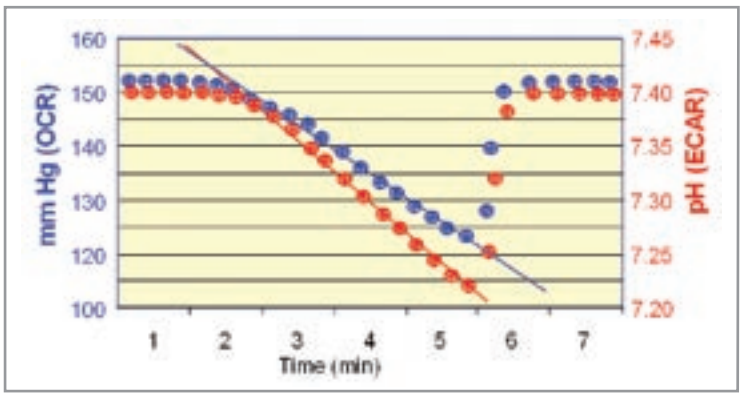
Flux Paks 2 analytes	75011
Flux Paks 3 analytes	75012
XF Assay Medium	75020
XF96 FluxPaks	75013

Measuring Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR)

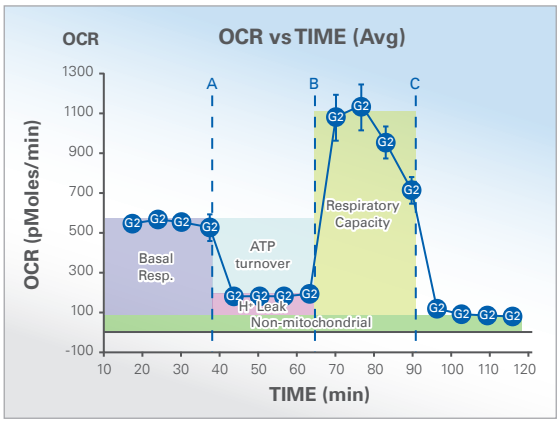




A temporary 7 µl microchamber is formed for the measurement.

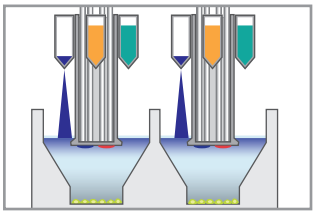


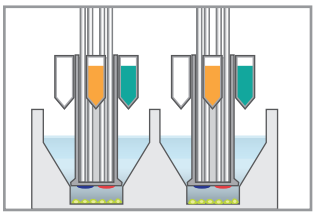
The rate is calculated from the slope

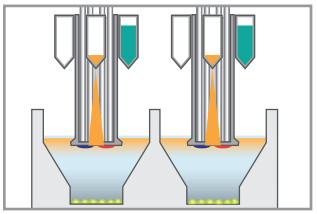


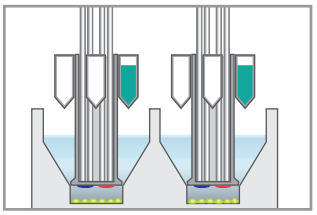
In this experiment with primary hippocampal neurons the bioenergetic profile was determined, starting with basal respiration. Then ATP turnover and the maximal respiratory capacity was determined.

	OCR	ECAR	CDPR	Injection ports	Working volume
XF24	✔	✔		4	0.5-1.2 ml
XF24/3	✔	✔	✔	4	0.5-1.2 ml
XF96	✔	✔		2	0.2-0.45 ml


injection compound A


measurement


injection compound B


next measurement

The flux packs which are required for the assays consist of a disposable 24-well or 96-well, dual-analyte sensor cartridge, a calibration plate, and a lid. The measured analytes are OCR (oxygen consumption rate) and ECAR (extracellular acidification rate). Two fluorophores for analyte detection are embedded in a polymer spotted on the end of each of the 24 or 96-well sensor sleeves.

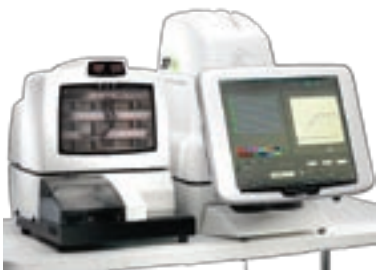
Each sensor cartridge also contains a unique multi-well drug delivery system, which injects 25 to 100 µl of up to four different drug compounds into each cell well on command.

The Extracellular Flux System


XF Assay Kits


XF Cell Culture Plates


XF24 Analyzer
XF24-3 Analyzer


XF96 with Prep Station

EVOS Microscopes

EVOS
The Microscope Evolved.

EVOS is a fully integrated digital imaging system that combines an inverted microscope, a high-resolution digital camera, and a 15" LCD display instead of using traditional oculars. EVOS' novel, user-oriented design provides top-quality imaging combined with unprecedented ergonomic features. The microscope is compact, portable and requires no external computer for image capture. Pictures are stored directly on an USB-drive.

You can view, capture, and archive images of specimens in various culture vessels and on slides – all with the ease and comfort of the EVOS imaging system.

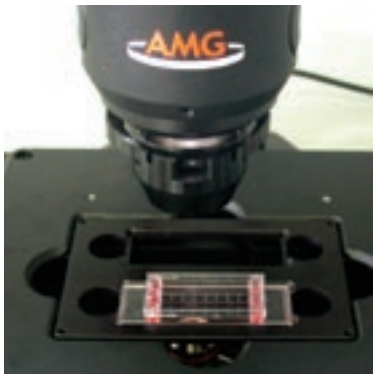
catalog numbers:

equipment

EVOS xl	73022
EVOS fl	73102

inserts

4 x µ-Dish 35 mm	73402
2 µ-Slides	73401
general	73409
2 x µ-Dish 50 mm	73403

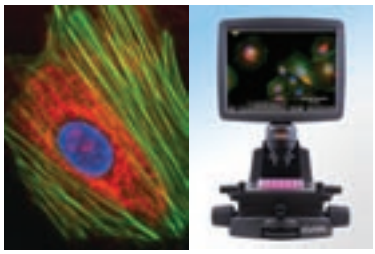


The EVOS$_{fl}$

In addition to the EVOS$_{xl}$ with support for phase contrast, AMG has also released the new fluorescence microscopy system, the EVOS$_{fl}$, designed to simplify workflow while delivering exceptional performance and reliability for fluorescence imaging.

The EVOS$_{fl}$ contains an embedded microprocessor, creating a high level of user functionality without an external computer. Users can set image acquisition modes, adjust exposure parameters, capture, overlay and export multi-channel fluorescence images. The software also monitors and reports on the filters and objectives that are in use at any time.

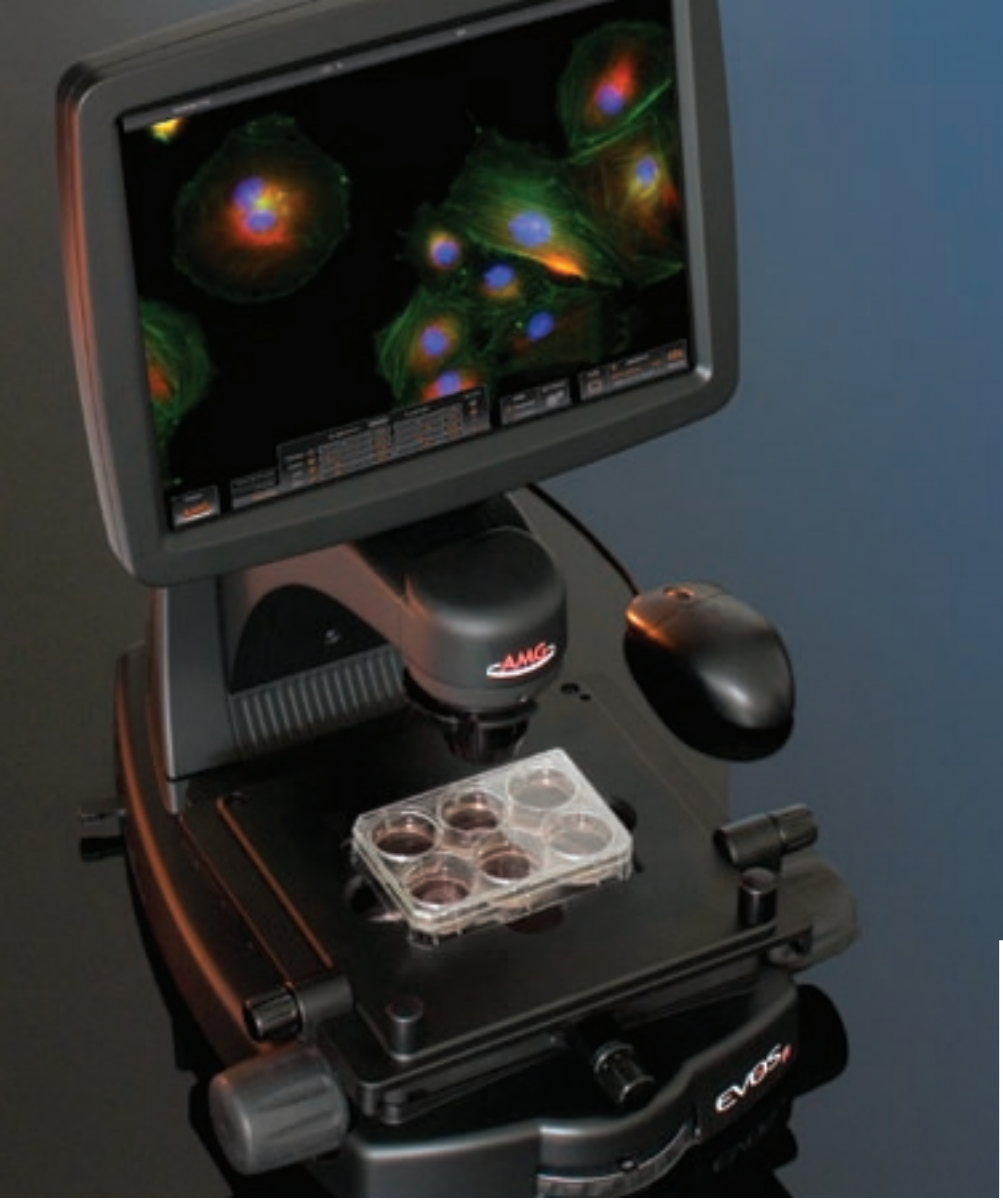
EVOS$_{xl}$ with Phase Contrast



EVOS$_{fl}$ with LED Fluorescence

EVOS systems: EVOS$_{xl}$ and EVOS$_{fl}$ in comparison

	EVOS$_{xl}$	EVOS$_{fl}$
Break free from the eyepiece • Adjustable 15" color LCD for viewing at various angles and distances • Comfortable viewing without back and neck strain • Ideal for meetings, presentations & training	✔	✔
Small footprint • The EVOS fits easily in tight spaces on a lab bench or inside a hood.	✔	✔
Plug and play • LED illumination: Instant-on, no warm-up, no cool-down • No adjustments, calibrations or assembly are needed • Stage inserts available for multiple types for vessels & slides	✔	✔
Multiuser discussion and teaching • The EVOS on-screen display makes it easy to present data to groups or discuss results with colleagues. • For large audiences, use the DVI output port to connect directly to a projector or external monitor.	✔	✔
Light cubes • Patented EVOS$_{fl}$ light cubes use LEDs that will last more than 50,000 hours, eliminating the need to change bulbs. • Support for up to 4 fluorescent channels (3 standard) with manual switching • GFP, RFP, & DAPI light cubes • YFP, CFP, Cy5, & Texas Red light cubes (optional) • Hard coated optical filters: reliable & durable		✔
Enjoy superior illumination, maintenance free • LED illumination • Constant, cool color temperature • Long service life (50,000 hrs)	✔	✔
Capture and document your work with digital tools		
• 1-click image capture	✔	✔
• 3-megapixel CMOS color camera	✔	
• High resolution monochrome CCD camera (Sony ICX285 chip)		✔
• 1-click image overlay mode		✔
• Onboard computer	✔	✔
• USB thumb drive data storage	✔	✔
The mechanical "glide" stage model for maximum efficiency • Moving stage allows sample to move without touching the vessel • Generous stage-travel range accommodates entire area of a microtiter plate	✔	✔
Objectives • 4x / 10x / 20x / 40x • 2x / 60x (optional)	✔	✔



EVOS™

The Microscope Evolved.

EVOS Microscopes

The EVOS microscopes are designed to simplify workflow while delivering exceptional performance and reliability for imaging. The line can also be combined with the ibidi heating stage and incubation unit.

Find more information on pages 108 - 109.

ibidi®

cells in focus

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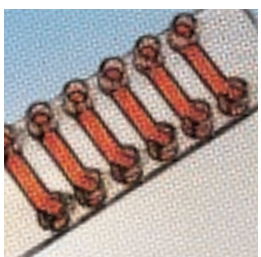

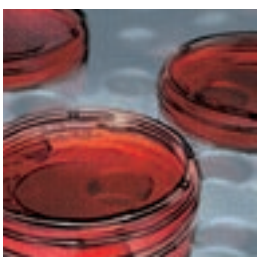

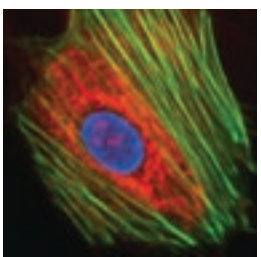

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