cells in focus ibidi®

ibidi Application Guide

Cell Culture and Preparation for Imaging

Selected Publications

Gstraunthaler G., Lindl T., Zell- und Gewebekultur, Allgemeine Grundlagen und spezielle Anwendungen, 7. Auflage, Springer Spektrum, Berlin, Heidelberg, 2013, 10.1007/978-3-642-35997-2

Freshney, R. I., Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications, 7th Edition, Wiley-Blackwell, Hoboken, 2016

E. Horn, R Zantl. Phase-Contrast Light Microscopy of Living Cells Cultured in Small Volumes. Microsc Anal, 2006, 20(3):5–7
read abstract

Cell Handling

Handling Cells in Culture

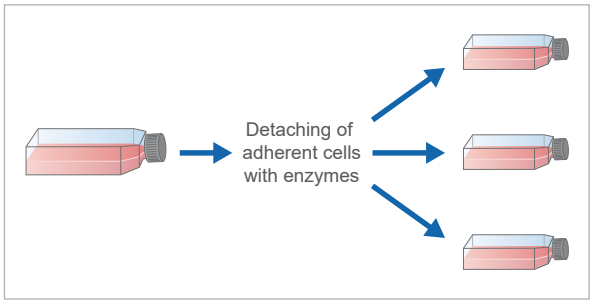
Starting at the end of the 19th century, scientists have been isolating cells out of their natural environments from various organisms (e.g., human, mouse, swine, chicken, and plants) and placing them in an *in vitro* (Latin "in the glass") culture. The experimental cells have been extracted from a number of different tissues and organs, such as intestine, kidney, blood, brain, mammary, and many cancer entities.

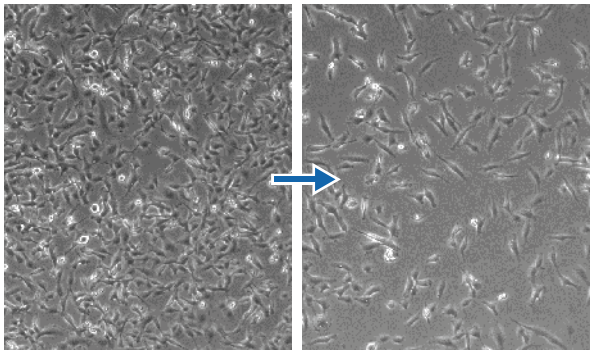
The aim of culturing cells *in vitro* is to mimic the *in vivo* (Latin "in the living") conditions inside an incubator or—when doing live cell imaging—even under a microscope. Every cell type requires tailored culture parameters for optimal health, viability, and growth. For all experiments, it is crucial to keep the conditions sterile, homogeneous and reproducible, in order to get comparable results.

Passaging Cells

In order to keep cells in a proliferative state, and to prevent them from reaching overconfluence, it is important to passage them at defined intervals. Cell passaging must be done regularly, in order to keep the cells in culture, achieve volume upscaling, or to seed them at the start of an experiment. This procedure is also called cell splitting or subculturing. Among other factors, the optimal time for passaging depends on the proliferation rate, as well as the required cell number. The exact splitting protocol is unique for every cell type. Generally, adherent cells are detached from the substrate using proteolytic enzymes (mostly trypsin/EDTA), which are necessary for the digestion of their protein attachment bonds. Next, the cells are homogenized, counted, and seeded into a new vessel, thus increasing the passage number by one.

Some cell lines, such as HeLa or other cancer cells lacking cell cycle regulation, can be passaged infinitively without losing their ability to divide (immortal cell lines). Also, this is the case for some embryonic cell types or pluripotent stem cells. Other cells, especially primary cells (e.g., isolated cells from adult organs), will lose this ability after a few passages, because they either undergo senescence, they differentiate, or they die. Therefore, it is important to use cells with a similar passage number for reproducible experiments.



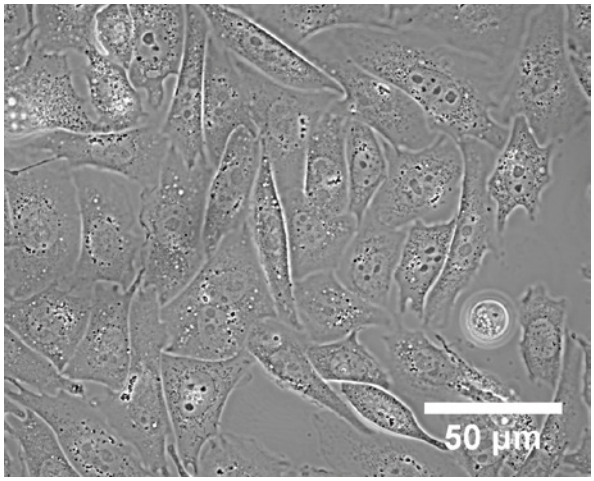


Passaging / subcultivation = transferring cells into fresh cultureware and growth medium

Read on and learn more about Parameters for Healthy Cells, Prevention of Contaminations, or Cell Preparation for Imaging.

Seeding Cells

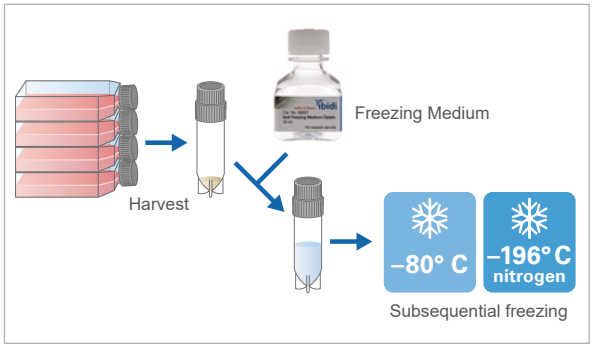
For cell seeding, choosing a density that is suitable for the respective cell type, cell culture vessel, and experimental setup is necessary. If the density is too low, the cells might stop growing due to an insufficient release of growth factors into the culture medium. Singular cells that lack cell-cell and cell-matrix communication might even die because of a special form of programmed cell death called anoikis. If the seeding density is too high, the cells might already reach overconfluence after a very short time. This leads to an impaired proliferation and cell detachment from the substrate, which results in non-reproducible experimental conditions.



Confluent cell layer of CHO-K1 cells

Freezing Cells

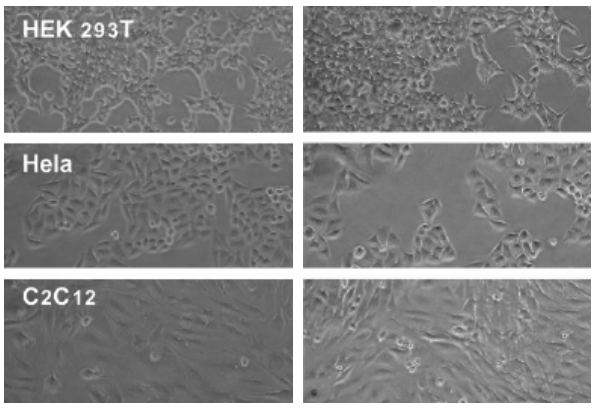
Standard cell types can be stored for short-term periods at –80°C, or long-term in liquid nitrogen. To prepare the cells for this, they need to be trypsinized, homogenized, and centrifuged before freezing them using a special freezing medium. To counter the cell stress caused by this process, it is recommended to use a freezing media with high recovery rates. In addition, the thawing procedure must be carried out quickly (e.g., in a water bath at 37°C), before transferring the cells to the culture medium and putting them into an incubator to create physiological conditions.



Cells can be stored for future use via freezing (= cryopreservation)

ibidi Solution

The ibidi Freezing Medium is ideally suited for direct freezing at –80°C, or when using liquid nitrogen. It is serum free, can be used with all standard cell lines, and has very high recovery rates.



Three different cell lines before freezing (left) and 12 months after (right). The cells were stored at -80°C. The cells were stored at -80°C.

Further reading:

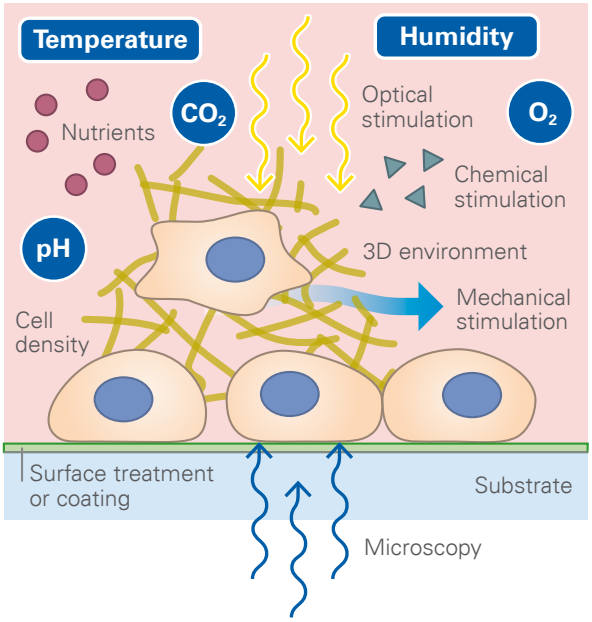
Gstraunthaler G., Lindl T., Zell- und Gewebekultur, Allgemeine Grundlagen und spezielle Anwendungen, 7. Auflage, Springer Spektrum, Berlin, Heidelberg, 2013, 10.1007/978-3-642-35997-2.

Freshney, R. I., Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications, 7th Edition, Wiley-Blackwell, Hoboken, 2016.

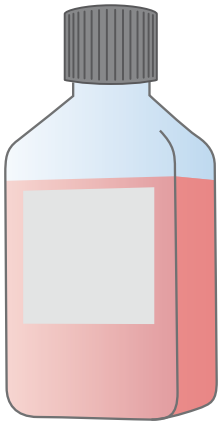
Parameters for Healthy Cells

Many factors determine the success of culturing cells by keeping them healthy and viable. Depending on the cell type and the experiment, these parameters must be determined and carefully supervised:

- Nutrient Supply
- Carbon Dioxide (CO_2) Levels and pH of the Medium
- Flow and Shear Stress
- Humidity and Evaporation
- Temperature
- Oxygen (O_2) Levels



Nutrient Supply



Cells need to be constantly surrounded by a suitable culture medium that supplies the cells with all the nutrients they need to be vital.

Many specialized media are available on the market for a variety of cells and experimental needs, such as Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) medium. Depending on the experiment and the cell type, the medium often has to be supplied with different substances (e.g., with fetal calf serum/fetal bovine serum (FCS/FBS) that delivers essential growth factors).

In order to guarantee optimal nutrient supply and constant environmental conditions, the medium needs to be changed at defined intervals (e.g., daily).

Carbon Dioxide (CO_2) Levels and pH of the Medium

A pH of 7.2–7.4 provides optimal growth conditions for most mammalian cells. The ambient carbon dioxide (CO_2) levels are an important parameter for cell culture, since they influence the pH of most cell culture media. They have to be kept as constant as possible within the incubator (5% CO_2 is the most commonly used concentration). Most commercial cell culture media contain phenol red as a color indication for the pH. If the color turns yellow (acidic) or purple (alkaline), the medium should be replaced.

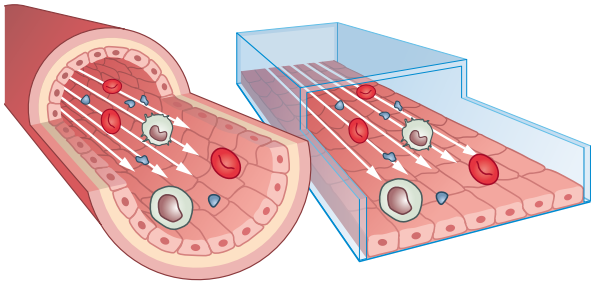
Find more details about how CO_2 levels influence the pH of the cell culture medium on our website.

Flow and Shear Stress

Many cell types, such as endothelial or epithelial cells, are in contact with moving fluids. This liquid flow causes shear stress, a mechanical force that influences the cell morphology, cell polarization, and cell behavior in many ways.

When cells are cultured without flow, shear stress-dependent cellular changes cannot be considered. Cell culture under flow simulates this mechanical stimulus, and induces a more physiological, *in vivo*-like behavior.

Further mechanical influences that should be considered when working with cells are compression (e.g., in cartilage cells) or strain.



Get more information about how to plan and perform a flow experiment in our Application Chapter on our website, or download the whole "Cell Culture Under Flow" Application Guide as a PDF here.

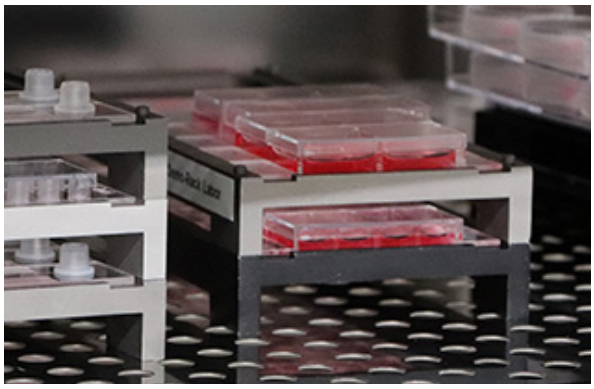
ibidi Solution

The ibidi Pump System allows for the defined shear stress simulation of various physiological conditions (e.g., continuous unidirectional, oscillating, or pulsatile flow). Combined with the ibidi Channel Slides, it is ideally suited for live cell imaging of cells under flow and immunofluorescence for analyzing shear stress response.

Humidity and Evaporation

Constant levels of salts, nutrients, and other cell culture medium components are essential for maintaining reproducible cell behavior. They are also crucial for consistent and reliable *in vitro* results. Any evaporation from the cell culture vessels increases the substance concentration in the medium in an undefined way.

Cell culture vessels in the CO_2 incubator are normally cultivated in an open system (e.g., gases can diffuse through the cell culture plastics), and are therefore in an equilibrium with the surrounding air. In standard CO_2 incubators, the humidity is created by evaporation from a water tray. Optimally, the relative humidity (RH) within an incubation system should be within the range of 90%–95%.



When small cell culture vessels with low volumes are used, it might be advisable to place them in a wet chamber.

ibidi Solution

ibidi's Patented Humidity Control

The ibidi Humidity Control ensures a constant and very high relative humidity (RH) level inside the ibidi Stage Top Incubator—identical to the conditions in standard cell culture incubators. This unique and patent-protected technology actively humidifies the gas mixture in a fast and reliable way before it enters the Stage Top Incubator. Learn more about ibidi's unique kind of Humidity Control on our website.

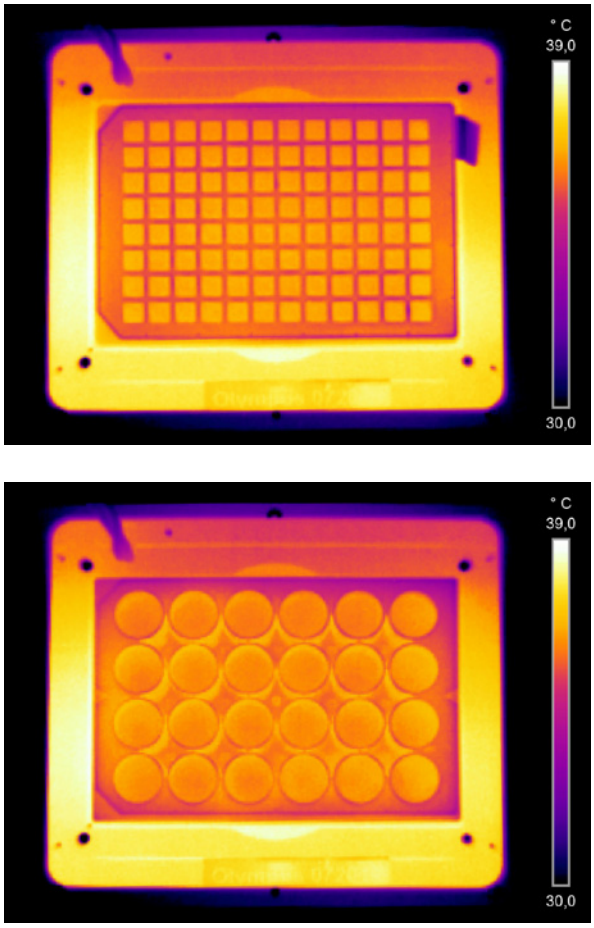
Temperature

The environmental temperature strongly influences the metabolism and the activity of cells. Temperature changes affect cell adhesion, protein expression, proliferation, and many more cellular parameters. A temperature of 37°C is optimal for mammalian cell culture, including human, mouse, and rat cells. Cells from non-mammalian organisms, such as chick or yeast, require different temperatures. Plant, fish, and insect cells can be cultured at room temperature.

Because most cell types do not tolerate long changes in temperature, all working steps in the hood have to be carried out as quickly as possible (while maintaining sterility and precision). In addition, cell culture media and reagents need to be prewarmed before use (e.g., in a water bath).

ibidi Solution

The ibidi Stage Top Incubation Systems provide a stable and homogeneous temperature for live cell imaging experiments. The temperature is smoothly changeable and can easily be adapted to the requirements of the investigated cell type.



Stable and consistent temperature distribution in every well of multiwell plates in the ibidi Stage Top Incubation System. Images were acquired with a FLIR thermal camera.

Oxygen (O_2) Levels

The O_2 concentration is another important parameter that should be controlled in cell culture assays, particularly when working with tumor cells, stem cells, or when analyzing the effects of hypoxia. The *in vivo* conditions of more or less all cell types have oxygen concentrations that are far below the oxygen concentration in ambient air.

ibidi Solution

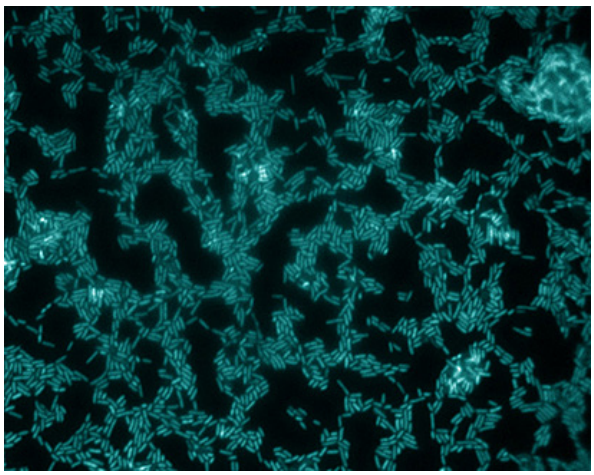
For hypoxia assays, the CO_2/O_2 versions of the ibidi Stage Top Incubation Systems allow for the precise control of the oxygen levels in your cell culture vessel. The ambient O_2 level can be controlled within a range of 1%–21%, and can easily be adapted to the experimental requirements.

To make accurate O_2 measurements, we recommend using one of the systems from ColibriPhotonics and preSens GmbH.

Learn more about the importance of oxygen levels in cell culture assays on our website.

Prevention of Contaminations

Contaminations are a serious and frequent issue in cell culture that absolutely has to be avoided. They can lead to false results, and at some stage they can irreversibly and completely destroy the cells in culture. The physiological temperature and humidity in the incubator, as well as the nutrients in the medium, provide excellent conditions for the growth of contaminating microorganisms, such as bacteria, mycoplasma, fungi, and yeast. Also, cross-contamination, which is the unwanted introduction of foreign cells into an existing culture, is a problem that must be taken seriously. If it remains undiscovered, a researcher could work with an entirely different cell line than the initial one without noticing, which makes all results of cell culture assays useless.



Sterility Guidelines

For reproducible experiments and unrestricted cell culture, all possible sources of contamination must be eliminated by maintaining the highest possible level of sterility. This is achieved by following these rules:

- Keep up an aseptic working technique
- Sterilize all media supplements and equipment (e.g., by autoclavation or filtering)
- Disinfect all surfaces (e.g., with 70% isopropanol or ethanol), especially under the hood
- Keep the surfaces free of waste and unnecessary items
- Keep the water bath and the water inside the incubator clean (if necessary, add a decontamination agent)
- Regularly clean the incubator
- Regulate the number of people in the room and use separate clothing and gloves in the cell culture laboratory
- Avoid coughing, sneezing, or talking (humans are the most frequent source of contamination)
- Bind your hair and do not touch your face during cell handling
- Put all cells from new sources into quarantine and perform a quality control
- Examine media and vessels daily for contamination
- Handle only one cell line at a time, and use your own medium to prevent cross-contamination
- Establish a mycoplasma testing routine for all cultures (e.g., by PCR) to prevent mycoplasma contamination

Use of Antibiotics

To protect against bacterial contamination, the antibiotics penicillin and streptomycin can be added to the cell culture medium. However, working without antibiotics increases the validity of the results, because the continuous use of antibiotics can tempt to non-sterile work. Furthermore, contaminations might be hidden and resistances could develop. To circumvent these pitfalls, it is advisable to culture the cells for 2–3 weeks without antibiotics from time to time.

Testing for Contamination

While contamination with microorganisms such as bacteria or fungi is immediately noticeable under the microscope, contamination with other microorganisms such as mycoplasma can remain undiscovered, if not specifically tested for. Mycoplasma-contaminated cell cultures are often infrequent, but may show undesirable functional changes in experiments. For the testing of such microbes, various companies offer kits based on PCR or ELISA.

Cell Preparation for Imaging

A well-planned experimental setup, as well as the optimized, reproducible handling of cells before imaging, are the keys for a successful microscopy experiment. To this end, several factors must be considered, and these depend on the microscopy method and the general setup being used:

- Vessel Geometry
- Sterile and Reproducible Cell Handling
- Substrate, Surface, and Coating
- Viable and Healthy Cells
- Meeting Optical Requirements for Microscopy
- Optional: Meeting Live Cell Imaging Requirements

What are the crucial questions for planning an experiment?

Are you choosing the optimal vessel geometry for your experiments and microscopy technique?

A large variety of cell culture vessels exist to meet the complexity of diverse cell culture assays, such as experiments using suspension or adherent cells, 2D or 3D assays, single or co-culture assays, and many more. The main culture vessel geometries (e.g., growth area and volume) are important factors that affect the outcome and interpretation of an experiment, and should be carefully considered before starting. Additionally, the vessel geometries influence the physical (e.g., evaporation) and optical (e.g., meniscus effect) properties in the experiments.

ibidi Solutions

We provide imaging chambers with various geometries that are specifically tailored to your assay. Get more detailed information in our application chapter, "The Geometry of the ibidi Chambers".

Do you continuously take care of sterile and reproducible cell handling?

For all experiments, it is crucial to keep the conditions sterile, homogeneous and reproducible, in order to get comparable results.

Read on and learn more details about Cell Handling and Prevention of Contaminations.

Did you choose a suitable substrate, surface, and coating for your specific cell type and microscopy method?

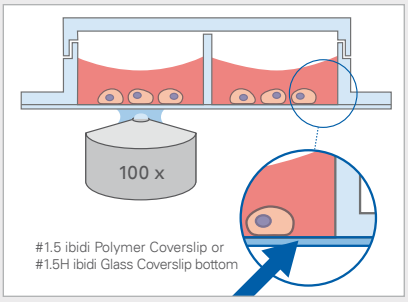
The growth, development, and signaling of cultured cells strongly depends on the surface being used for cell seeding. Additionally, the optical specifications strongly determine which substrate is advisable. Borosilicate glass is widely used as a substrate, however, a coating might be necessary to support cell adherence. The use of polymers is also very popular. They are usually tissue culture-treated to allow for direct cell adherence; however, not all polymers available on the market are suitable for microscopy. Labware with the ibidi Polymer Coverslip bottom provides the highest optical quality for various high-resolution microscopy applications.

However, if the cells require a specific extracellular matrix (ECM) on the surface, then coatings such as collagen I, collagen IV, or poly-L-lysine can be used. For suspension cells or spheroid culture, a non-adherent surface, such as ibidi Bioinert, should be used.

Also, it should be carefully considered whether a 2D or a 3D experiment will be performed. For more details, read our extensive Application Chapter about 3D Cell Culture Assays.

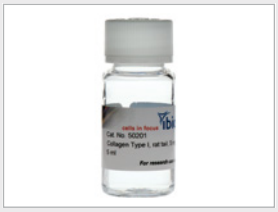
ibidi Solutions

ibidi Labware



The outstanding characteristic of ibidi µ-Slides, µ-Dishes, and µ-Plates is their thin coverslip bottom, which is excellent for high-end microscopy applications. ibidi offers labware with the ibidi Polymer Coverslip bottom or the ibidi Glass Coverslip bottom. We provide imaging chambers with various surfaces and coatings, for example, the ibiTreat (tissue culture-treated) surface for the direct culture of adherent cells. Get more detailed information in our application chapter, "Surfaces and Coatings".

Collagen Type I, Rat Tail

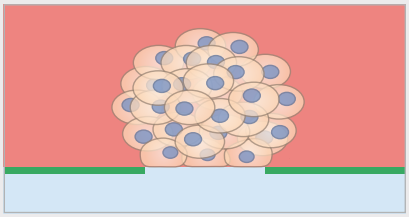


The ibidi Collagen Type I, Rat Tail is a non-pepsinized, native collagen for modeling ECM in gel matrices. Its fast polymerization facilitates optimal cell distribution in 3D gels. In the ibidi Application Note Cell Culture Coating (AN 08) (PDF), you will find detailed information on how to do your own coating onto µ-Slides using collagen I, collagen IV, fibronectin, poly-L-lysine, or poly-D-lysine.

Non-Adherent Bioinert Surface

Bioinert is a stable, biologically inert surface made for long-term culture and high-resolution microscopy of organoids, spheroids, and suspension cells on a non-adherent surface without any cell or biomolecule adhesion. It is currently available as the µ-Dish 35 mm, high Bioinert.

Micropatterning

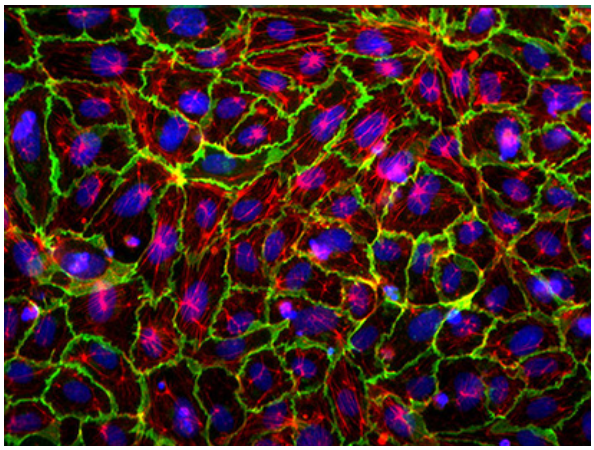


The ibidi µ-Patterning technology enables spatially defined cell adhesion for various 2D and 3D cell culture applications. Miniaturized adhesive patterns (e.g., lines, squares, or dots) are irreversibly printed on the non-adhesive Bioinert surface of the ibidi Polymer Coverslip, allowing for precisely controlled cell adhesion.

Are you ensuring that the cells are viable and healthy when they are being cultured, as well as throughout the whole imaging procedure?

Microscopy can be done with both living and fixed cells, depending on the experimental setup and the required readout. In living cells, the microscopy procedure causes stress. The cells have to be taken out of the incubator, which leads to a reduction in the surrounding temperature and humidity. Additionally, the cells are exposed to light for a certain amount of time, which might cause phototoxicity. Generally, all microscopy steps should be carried out as quick as possible—while maintaining precision—to minimize cell stress.

Therefore, only viable and healthy cells should be used for any imaging experiment. To ensure this, cell attachment, morphology, confluency, nutrient supply, and signs of contamination should be controlled daily under the microscope. Plus, several parameters, such as temperature and humidity, must be kept consistent.



Immunofluorescence of Human Umbilical Vein Endothelial Cells (HUVEC) in a µ-Slide 4 Well

Read more about how to prevent phototoxicity in our application chapter, "Image Acquisition", or learn more about Cell Handling or Parameters for Healthy Cells.

Can you ensure that the optical requirements for your microscopy technique of choice are being met?

Every microscopy technique has its own special requirements for the substrate and cell pretreatment, and these should be considered when planning the experiment. You can get more detailed information about the optical parameters of the different substrates in our application chapters, "Microscopy Parameters of Materials" and "Comparison of Material Specifications".

Furthermore, some general recommendations apply before starting microscopy:

- All media should be equilibrated in order to prevent the formation of air bubbles inside the vessels. By minimizing temperature differences, external convective currents can be avoided that can considerably disturb microscopy.
- When performing fluorescence microscopy, it is especially crucial to minimize cell stress caused by excitation light, because this might alter the outcome of an experiment. Please get more detailed insights about provisions against phototoxicity on our website.
- Also, autofluorescence of cell culture vessels or media can be an unwanted side effect. Therefore, specialized cell culture vessels with low autofluorescence should be applied for fluorescence microscopy.

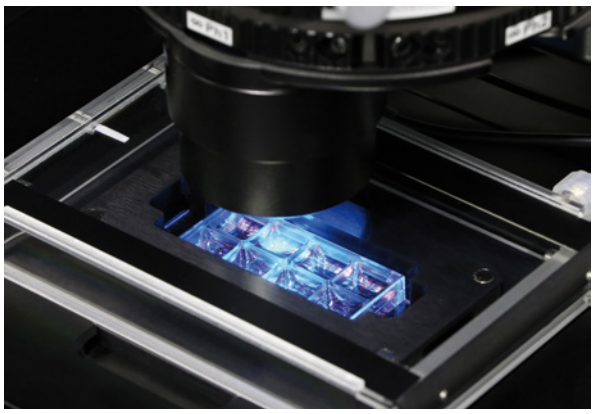
ibidi Solutions

Many microscopy techniques, such as phase contrast, widefield fluorescence, and confocal microscopy, are possible without restrictions when using any of the ibidi labware that contain the ibidi Polymer Coverslip bottom or the ibidi Glass Coverslip bottom.

Get more detailed information about the ibidi imaging chambers and different microscopy techniques in our application chapter, "Microscopy With ibidi".

When planning a live cell imaging experiment, does your setup meet all the necessary requirements?

If several images in a row, or at specific time points, are needed, then live cell imaging might be the best choice for you. To keep the cells alive and healthy during a live cell imaging experiment, physiological conditions must be established and maintained on the microscope—the same set up as inside the incubator.



ibidi Solution

The ibidi Stage Top Incubation Systems are available for slides/dishes and for multiwell plates. They allow for the accurate and reliable control of important live cell imaging parameters (temperature, CO_2 and O_2 concentration, and relative humidity) during short-term and long-term assays on any inverted microscope. If an additional control of the oxygen level is needed (e.g., in hypoxia experiments), the ibidi Stage Top Incubation Systems, CO_2/O_2 are the ideal solution.

Get more information on how to plan and perform a live cell imaging experiment in the Application Chapter on our website, or download the whole "Live Cell Imaging" Application Guide as a PDF here.

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