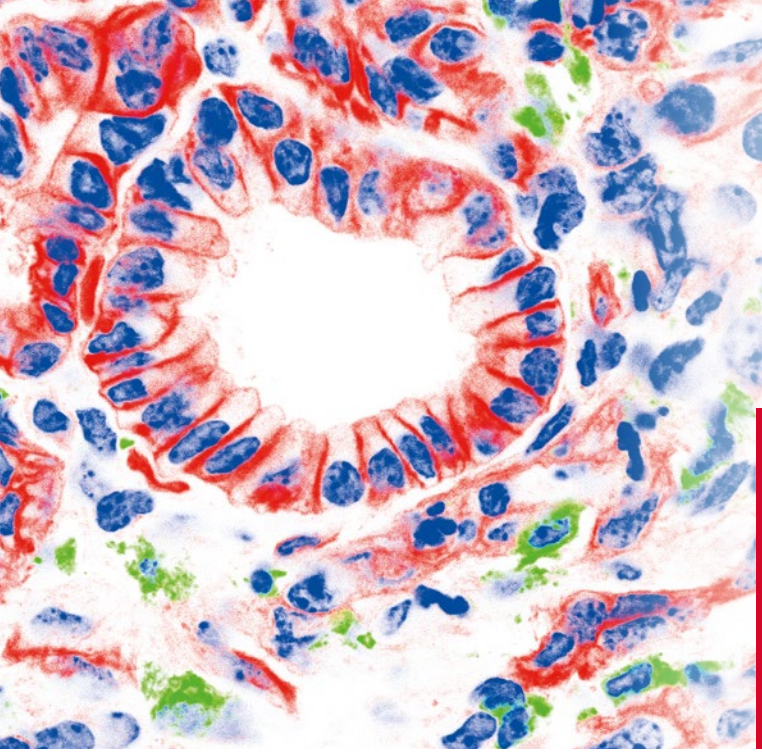


cells in focus

ibidi®

ibidi Application Guide

Cancer Research

Selected Publications

Jeffries, G.D.M., et al. (2020). 3D micro-organisation printing of mammalian cells to generate biological tissues. Scientific Reports. 10.1038/s41598-020-74191-w
Read article

Lüönd, F., et al. (2021). Distinct contributions of partial and full EMT to breast cancer malignancy. Developmental Cell. 10.1016/J.DEVCEL.2021.11.006
Read article

Xu, Z., et al. (2021). Endothelial deletion of SHP2 suppresses tumor angiogenesis and promotes vascular normalization. Nature Communications. 10.1038/s41467-021-26697-8
Read article

Newton, H.S., et al. L. (2020). PD1 blockade enhances K^+ channel activity, Ca^{2+} signaling, and migratory ability in cytotoxic T lymphocytes of patients with head and neck cancer. Journal for Immunotherapy of Cancer. 10.1136/JITC-2020-000844
Read article

Yang, L., et al. (2020). DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature. 10.1038/s41586-020-2394-6
Read article

Cancer:
Definition, Facts, and Hallmarks

Cancer is a large, diverse group of diseases. It is defined by uncontrolled cell growth anywhere in the body, with the potential to spread ("metastasize") to other organs. In 2020, an estimated 19.3 million new cancer cases and almost 10.0 million cancer deaths occurred worldwide. The most commonly diagnosed cancer is breast cancer, followed by lung, colorectal, prostate, and stomach cancers. In many countries, cancer ranks among the most common causes of death, and the numbers are increasing anually (Sung *et al.*, 2021). This illustrates the emerging need to develop novel therapies, which can only be realized by increasing the knowledge about the highly complex fundamentals of neoplastic diseases.

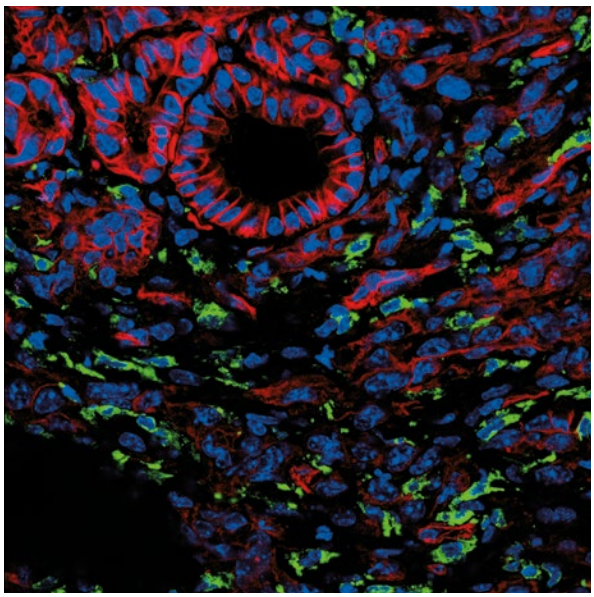
Tumors can develop almost everywhere in the human body and have many different manifestations. In spite of this diversity, some characteristics apply to all types of cancer, such as the uncontrolled proliferation of cells and their potential to metastasize to other parts of the body. Whereas cancer is relatively rare in children (with leukemia as predominant childhood cancer), increasing age is a clear risk factor for being affected. Further general risk factors are genetic predisposition and various environmental effects (e.g., smoking as one of the leading causes of lung cancer and ultraviolet light for skin cancer).

Some tumor types are very aggressive: pancreatic cancer (i.e., pancreatic ductal adenocarcinoma, PDAC), has a high metastasizing rate and only a few therapeutic options, resulting in 5-year-survival rates below 8% (Orth *et al.*, 2019). Other types have a very positive overall outcome: basalioma (basal cell carcinoma, BCC), which is the most common type of skin cancer, only rarely metastasizes and, in most cases, can be completely excised by surgery (Rogers *et al.*, 2015).

During the process of tumor development, cancer cells acquire defined biologic capabilities. They resist cell death, induce angiogenesis, sustain proliferative signaling, evade growth suppressors, activate invasion and metastasis, and enable replicative immortality—all these processes were published as the "Hallmarks of Cancer" back in 2000 by Hanahan and Weinberg. A decade later, these hallmarks were complemented by further hallmarks (avoiding immune destruction and deregulating cellular energetics), as well as the "enabling characteristics" (tumor-promoting inflammation as well as genome instability and mutation).

With their enormous work in basic and applied cancer research, thousands of scientists contribute every day to deciphering cancer mechanisms. ibidi helps these researchers develop therapeutics against this dismal disease with a variety of tailored solutions for cell-based assays and high-end microscopy.

For a better life without cancer!



Confocal microscopy of tumor-associated macrophages (GFP, green) infiltrating the pancreatic tumor tissue (Tomato, red). Nuclei are stained with DAPI (blue). Tissue sample of an endogenous pancreatic cancer mouse model with a double-fluorescent reporter gene to target macrophages. Data by Stefanie Bärthel, Translatum, Technical University of Munich, Germany.

Sung, H., et al. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 10.3322/caac.21660
Read article

Orth, M., et al. (2019). Pancreatic ductal adenocarcinoma: biological hallmarks, current status, and future perspectives of combined modality treatment approaches. Radiation Oncology. 10.1186/S13014-019-1345-6
Read article

Rogers, H.W., et al. (2015). Incidence Estimate of Nonmelanoma Skin Cancer (Keratinocyte Carcinomas) in the U.S. Population, 2012. JAMA Dermatology. 10.1001/JAMADERMATOL.2015.1187
Read article

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. Cell. 10.1016/j.cell.2011.02.013
Read article

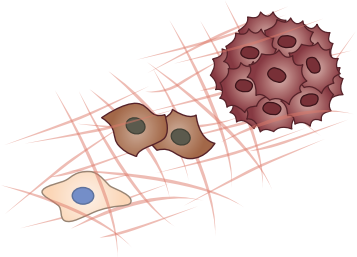
How ibidi Contributes to Cancer Research

ibidi develops solutions and products that facilitate a variety of cell culture assays covering the different aspects of cancer development and the metastatic cascade.

More than 16000 cancer-related publications using ibidi products show the outstanding contribution of ibidi to the large field of cancer research.

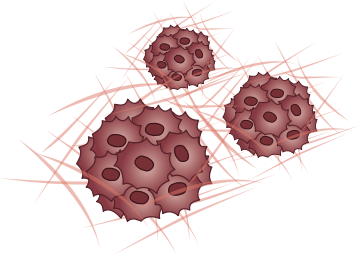
Various *in vitro* approaches, such as migration assays, chemotaxis assays, angiogenesis assays, or perfusion assays, will help you analyze the individual steps of cancer and metastasis. In addition, we offer detailed solutions for the complete workflow of your cell-based assay, from sample preparation to image analysis, to get you reproducible and valid results.

Learn more about ibidi solutions in your field of interest here:



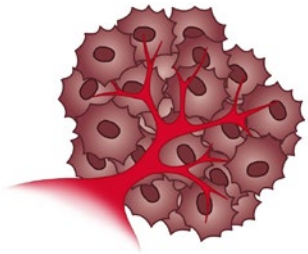
Tumor Cell Analysis

- Cancer Signaling Pathways
- Cell Fitness
- Proliferation
- Cell Death



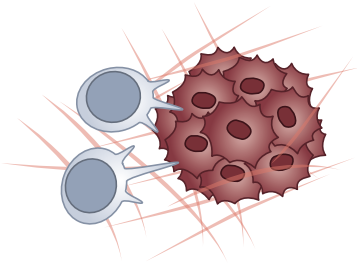
3D Models of Cancer

- Spheroids
- Organoids
- Single Cells in a Matrix



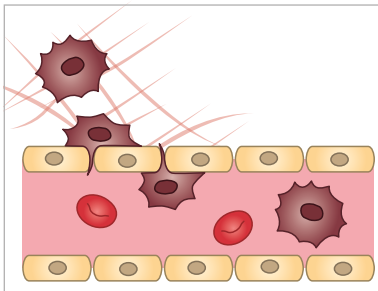
Tumor Vascularization

- Angiogenesis
- Chemotaxis



Immunooncology

- Tumor Microenvironment
- Hypoxia
- T Cell Killing Assays



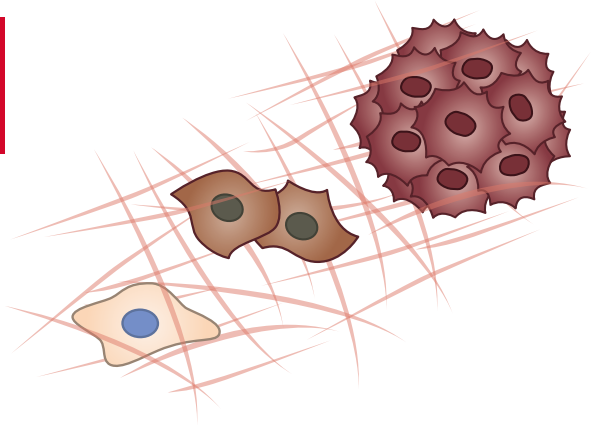
Invasion and Metastasis

- Tumor Cell Migration
- Chemotaxis
- Cancer Cells Under Shear Stress

ibidi Blog Article:

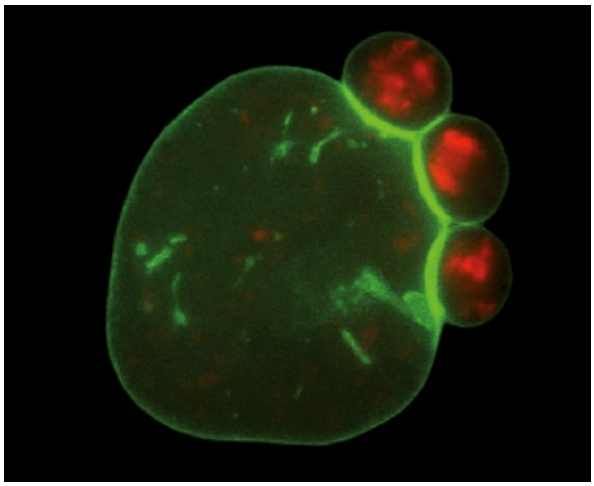
Want to know insights on cancer-related cell culture experiments? Raúl Peña (IMIM, Barcelona, Spain) communicates this topic in his article "The Patient in Focus: How to Use Cell Assays for the Discovery of New Cancer Therapies" in the ibidi Blog.

Tumor Cell Analysis



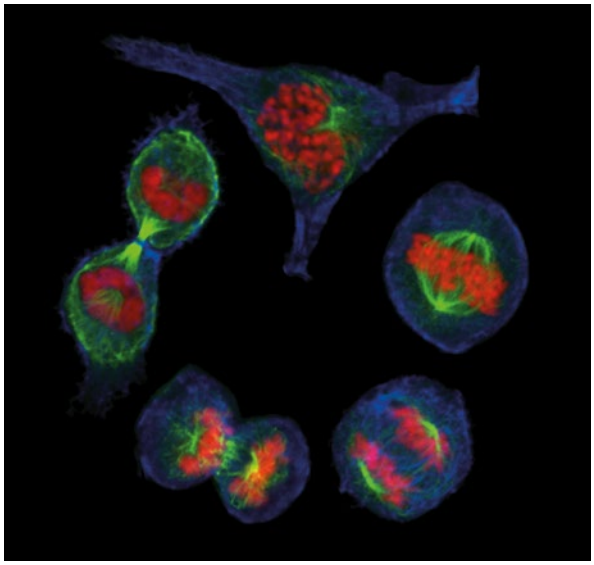
The emergence of cancer and metastasis is orchestrated by a vast and complex network of cancer signaling pathways. Depending on the tumor type, the expression and the mutational state of specific oncogenes (e.g., Ras, PI3K, and Myc), tumor suppressor genes (e.g., PTEN, P53, and Rb), and their downstream targets can give important information about the expected patient outcome. To test if a drug is a promising therapeutic, or to analyze the relevance of a gene for a defined tumor type, drug screens or gene knockdown/overexpression experiments are widely performed in cell-based models.

Analyzing the fitness, morphology, and behavior of tumor cells by live cell imaging gives first insights into the efficacy of drugs, antibodies, and other agents as potential novel therapies. In addition, the cellular expression and localization of the encoded proteins of interest after such treatments can be visualized by immunofluorescence staining and high-resolution fluorescence microscopy.



A paw inside the cell. A human bone osteosarcoma (U2OS) cell expressing GFP-tagged nuclear lamina (green) and RFP-tagged alternative splicing factor (red) undergoing nuclear blebbing. The cells were cultured and imaged in an ibidi µ-Dish 35 mm, high Glass Bottom. Data by Mohammad Amin Abolghassemi Fakhree, MESA+ Institute, University of Twente, Enschede, The Netherlands.

Cell proliferation, cell death, cell survival, and many more aspects can be measured using cell-based assays to serve as readouts for cancer cell fitness. In addition to standard 2D cell assays, cells can be cultured in a three-dimensional setup (e.g., creating spheroids or seeding single cells in a gel matrix). This way, the cellular microenvironment can be mimicked, closely representing the physiological situation. If the initial *in vitro* results are promising, the agents can later be tested *in vivo* and, at best, finally translated into the clinic.



MCF7 breast cancer cells during the different stages of mitosis. Cells were grown on an ibidi Glass Bottom Dish 35 mm and stained with phalloidin (blue) to visualize F-actin, α-Tubulin for the mitotic spindle (green), and DAPI (red) for DNA. Cells were visualized on a NIKON A1R scanning laser confocal microscope using a 60x objective. Data by Olga Tapia, (Health Research Institute Valdecilla (IDIVAL)) and Almudena Medina (Dept. Anatomy & Cell Biology), University of Cantabria, Santander, Spain.

Download detailed Application Guides at: ibidi.com/IFGuide and ibidi.com/LiveImagingGuide

ibidi Solutions for Tumor Cell Analysis

The ibidi µ-Slides and µ-Dishes include different geometries that combine optimal conditions for everyday cell culture and functional cell-based assays. They are ideal for immunofluorescence, live cell imaging, and high-resolution microscopy. The ibidi labware is available with the ibidi Polymer Coverslip and the ibidi Glass Coverslip.

The ibidi channel slides, especially the µ-Slide VI$^{0.4}$, are particularly suitable for immunofluorescence stainings: Their geometry is ideal for the exact exchange of small medium amounts, which is necessary during immunocytochemistry stainings, making the channel geometry ideal for low-volume immunofluorescence assays.

The Chamber Slides, removable are ideal for both low- and high-throughput immunofluorescence experiments and are suitable for the long-term storage of samples that are mounted with a glass coverslip.

The ibidi µ-Plates are ideal for high throughput drug screening and large-scale knock-down and overexpression experiments with high-resolution microscopy as readout. These imaging plates are compatible with robotics and plate readers due to an ANSI/SLAS (SBS) standard format. The ibidi µ-Plates have 24, 96, or 384 wells and are available with the ibidi Polymer Coverslip and the ibidi Glass Coverslip with extremely low autofluorescence for undisturbed fluorescence microscopy.

The ibidi Mounting Medium and the ibidi Mounting Medium With DAPI for immunofluorescence have a very low autofluorescence, prevent photobleaching and allow the sample to be stored for several weeks on the µ-Slide without the need for additional coverslips.

The ibidi Stage Top Incubators provide physiological conditions for live cell imaging on every standard inverted microscope. They include CO_2 and O_2 control (e.g., for hypoxia experiments) and actively controlled humidity. They are available for single slides and dishes as well as for multiwell plates.

Selected References

Bioprinting of cancer cells in the µ-Dish$^{35\ mm,\ low}$ to establish a 2D tumor model including microenvironment

Jeffries, G.D.M., et al. (2020). 3D micro-organisation printing of mammalian cells to generate biological tissues. Scientific Reports. 10.1038/s41598-020-74191-w
Read article

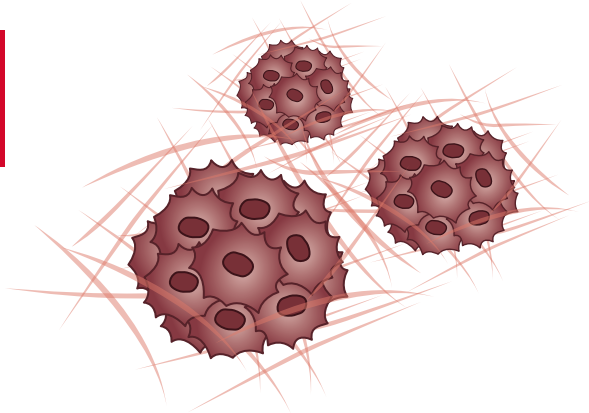
Live cell imaging of single dormant prostate cancer cells in the µ-Dish$^{35\ mm}$ Quad to analyze cell division

Yu-Lee, L.Y., et al. (2018). Osteoblast-Secreted Factors Mediate Dormancy of Metastatic Prostate Cancer in the Bone via Activation of the TGFβRIII–p38MAPK–pS249/T252RB Pathway. Cancer Research. 10.1158/0008-5472.CAN-17-1051
Read article

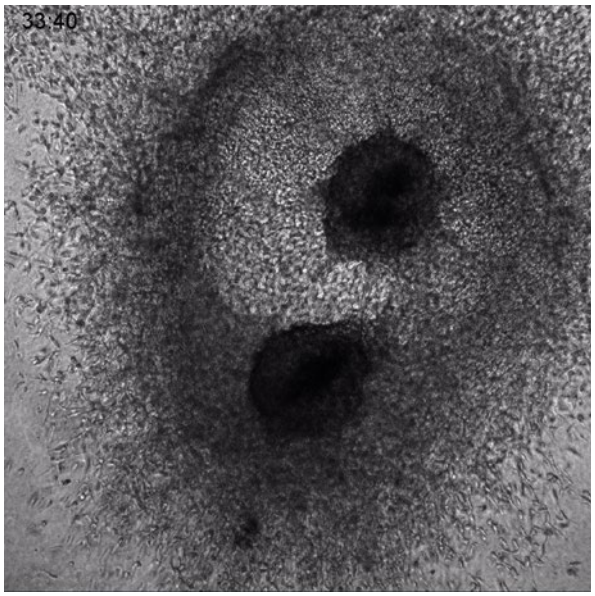
Quantification of circulating tumor cells in the µ-Slide 8 Well

Lüönd, F., et al. (2021). Distinct contributions of partial and full EMT to breast cancer malignancy. Developmental Cell. 10.1016/J.DEVCEL.2021.11.006
Read article

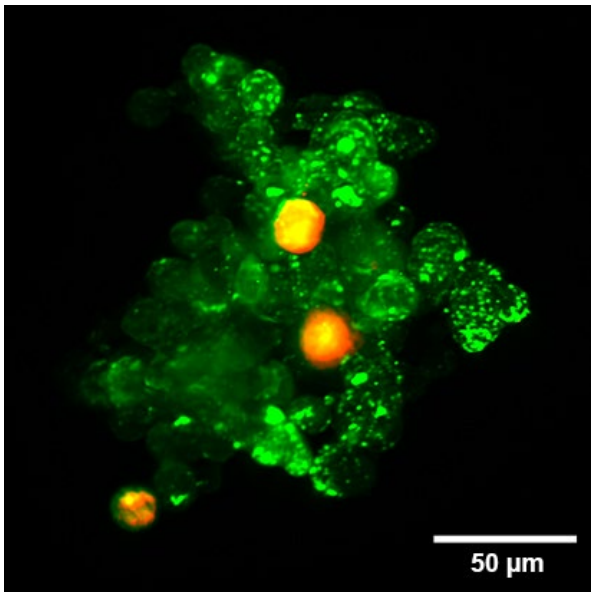
3D Models of Cancer



Tumor cells grow in a three-dimensional micro-environment, where they communicate and interact with each other and their surroundings. When cultured inside a 3D matrix, cells behave differently compared to a 2D environment. In many cases, a 3D cell culture setup more accurately reflects the *in vivo* situation. This includes drug screenings that use spheroids and organoids, which are indispensable nowadays as tumor models. This should be considered when analyzing cancer cell behavior, migration, proliferation, response to drug treatment, and gene and protein expression.



*Invasion of HT-1080 cancer cells in a 3D Collagen gel. Invasive human fibrosarcoma cancer spheroids (HT-1080) were embedded into **Collagen Type I, Rat Tail** gel. The invasion into the gel matrix was recorded for 48 hours in the **µ-Slide 8 Well**. 4x objective lens, brightfield.*



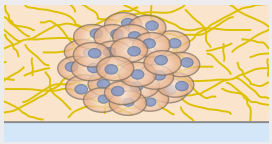
*Organoid co-culture of PDAC cells and Fibroblasts. Organoid co-culture of the human pancreatic cancer (PDAC) cell line PA-TU-8988T (green, stained with CellTracker™ Green) and the murine fibroblast cell line mPSC4 (red, stained with CellTracker™ Orange CMTMR) in the **µ-Slide Spheroid Perfusion**. The µ-Slide was covered with a 25 µm FEP foil for matching the refractive index closer to water during upright light sheet microscopy.*

The image was acquired by S. Volkery at MPI Muenster with the M Squared Aurora Airy beam upright light sheet setup. The sample was provided by K. Roth, University Marburg, Germany.

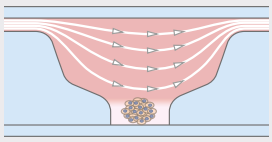
Download a detailed Application Guide at: ibidi.com/3DGuide

ibidi Solutions for 3D Cancer Models

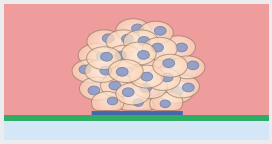
The ibidi Collagens Type I are non-pepsinized, native collagens for modeling ECM in gel matrices. Their fast polymerization facilitates optimal cell distribution in 3D gels. Read our Application Note about how to prepare a 3D gel using the ibidi Collagens Type I here: AN 26: Preparation of a Collagen I Gel.



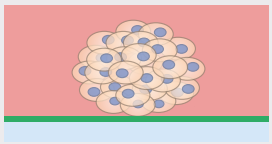
The µ-Slide Spheroid Perfusion is a specialized flow chamber for long-term spheroid culture. Each of the 3 x 7 wells forms its own niche, in which the specimen is cultured. The application of perfusion through the channel on top of the wells (e.g., by using the ibidi Pump System) ensures optimal nutrition and oxygen diffusion, without exposing the specimen to significant shear forces.



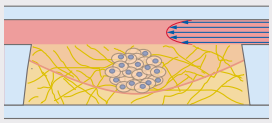
The µ-Slides With Multi-Cell µ-Pattern enable spatially defined cell adhesion for spheroid and organoid generation. Defined adhesion spots are able to catch all adherent single cells from a cell suspension. The surrounding Bioinert surface is fully non-cell-attachable. This forces all cells to aggregate to each other at the adhesion spots, thus forming spheroids in a defined and controllable way.



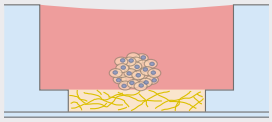
Bioinert is a stable, biologically inert surface for long-term culture and high-resolution microscopy of spheroids, organoids, and suspension cells on a non-adherent surface without any cell or biomolecule adhesion. It is available as µ-Dish$^{35\ mm,\ high}$ Bioinert, µ-Slide 8 Well^{high} Bioinert, and further products.



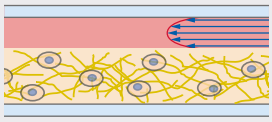
In the µ-Slide III 3D Perfusion, single cells, spheroids, or organoids can be cultivated in/on a gel layer or embedded in a 3D matrix. The channel geometry allows for superfusion with a low flow rate (e.g., with the ibidi Pump System), ensuring optimal oxygen and nutrient supply during long-term experiments.



The µ-Slide 15 Well 3D and the µ-Plate 96 Well 3D are cost-effective solutions for 3D culture and microscopy of single cells, co-cultures, spheroids, and organoids on/in gel matrices. The gel layer is directly connected to the medium reservoir above, enabling easy medium exchange by diffusion.



The µ-Slide I Luer 3D is designed for culturing cells on/in a 3D gel matrix with defined shear stress. The wells can be filled with a gel, in which cells can be embedded. For flow application, the channel on top can be connected to a pump (e.g., to the ibidi Pump System) to ensure optimal oxygen and nutrient supply.



Selected References

3D Sandwich culture of squamous cell carcinoma lines using the µ-Plate 96 Well 3D

Hoque Apu, E., et al. (2018). Desmoglein 3 – Influence on oral carcinoma cell migration and invasion. Experimental Cell Research. 10.1016/J.YEXCR.2018.06.037
Read article

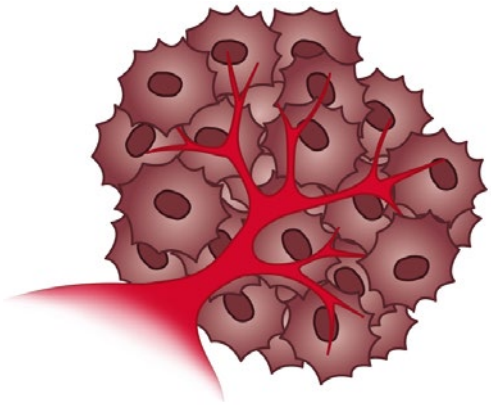
Breast tumor organoid culture in the µ-Slide 15 Well 3D

Lüönd, F., et al. (2021). Distinct contributions of partial and full EMT to breast cancer malignancy. Developmental Cell. 10.1016/J.DEVCEL.2021.11.006
Read article

Live cell imaging of HT29 tumor spheroid co-culture with neutrophils in the µ-Slide III 3D Perfusion

Teijeira, Á., et al. (2020). CXCR1 and CXCR2 Chemokine Receptor Agonists Produced by Tumors Induce Neutrophil Extracellular Traps that Interfere with Immune Cytotoxicity. Immunity. 10.1016/J.IMMUNI.2020.03.001
Read article

Tumor Vascularization

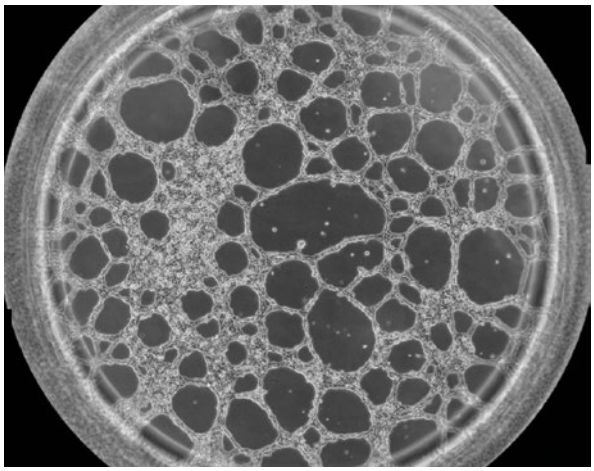


Tumor cells cannot survive without nutrients and oxygen. Therefore, cancer growth beyond a limited size needs to be accompanied by vascularization, which is induced by a variety of molecular mechanisms.

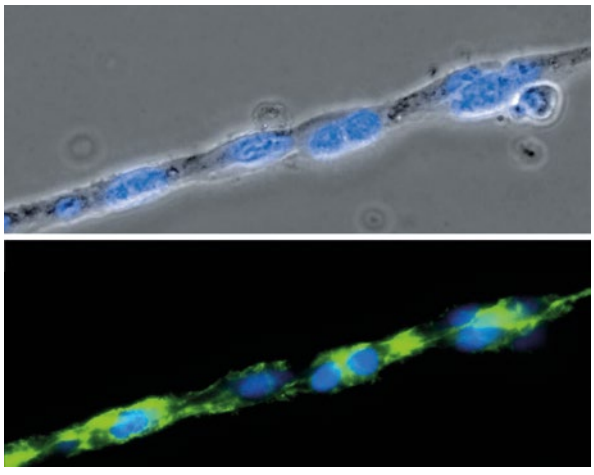
Already early during cancer progression, angiogenic processes are activated when cancer cells become hypoxic, because they are not connected to the blood circulation system. In response to hypoxia, tumor cells and surrounding stroma cells begin to secrete pro-angiogenic growth factors such as VEGF, which induces blood vessel formation through several mechanisms. This "angiogenic switch" allows tumors to grow and proliferate without the initial limitation. Apart from oxygen and nutrient delivery, newly formed vessels also provide waste transport and promote metastasis by facilitating the dissemination of cancer cells to distant sites. Therefore, it is not surprising that much effort has been invested in the research of anti-angiogenic therapies, many of which have been approved for several types of cancer. However, the success of these treatments has been only moderate so far.

Since tumor vascularization is an important process during cancer progression and tumor maintenance, which can also serve as a therapeutic target, **angiogenesis assays** are a widely used tool in cancer research.

Lugano, R., Ramachandran, M., & Dimberg, A. (2020). Tumor angiogenesis: causes, consequences, challenges and opportunities. Cellular and Molecular Life Sciences. 10.1007/S00018-019-03351-7
Read article



Phase contrast image showing one well of the µ-Slide 15 Well 3D with HUVEC cells on Matrigel® after 12 hours of incubation during a tube formation assay.

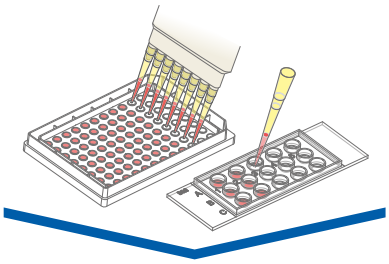


Phase contrast (top) and fluorescence microscopy (bottom) of a single strand composed of HUVEC cells during a tube formation assay in the µ-Slide 15 Well 3D. The F-actin cytoskeleton is stained green and the cell nuclei are stained blue.

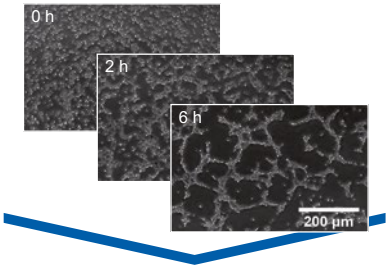
Download a detailed Application Guide at:
ibidi.com/AngioGuide

ibidi Solutions for Angiogenesis Assays

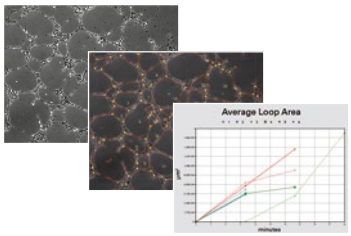
Sample Preparation



Live Cell Imaging



Data Analysis



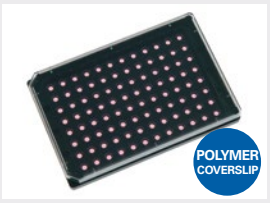
The µ-Slide 15 Well 3D and the µ-Slide 15 Well 3D Glass Bottom guarantee the convenient observation of tube formation on any inverted microscope without gel meniscus.

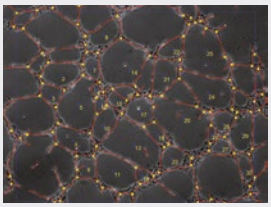
All common 3D gel matrices, such as the ibidi Collagens Type I, Matrigel®, and similar hydrogels, work within the ibidi angiogenesis assay. In addition, the slide's design helps to reduce costs drastically by minimizing the amount of gel needed to only 10 µl.

For large-scale angiogenesis experiments, we provide the µ-Plate 96 Well 3D.

The ibidi Stage Top Incubators provide physiological conditions for live cell imaging on every standard inverted microscope and are ideal for angiogenesis assays over longer periods of time. They include actively controlled humidity as well as CO_2 and O_2 control. They can be used with single slides (e.g., the µ-Slide 15 Well 3D) as well as with multiwell plates (e.g., the µ-Plate 96 Well 3D)

Tube Formation FastTrack AI Image Analysis is an AI-based automated image analysis solution for tube formation assays, which provides objective and reproducible results within minutes.





Selected References

Tube formation assay to analyze angiogenesis in triple-negative breast cancer using the µ-Plate 96 Well 3D

Wang, Y., et al. (2020). LncRNA-encoded polypeptide ASRPS inhibits triple-negative breast cancer angiogenesis. Journal of Experimental Medicine. 10.1084/JEM.20190950/132618
Read article

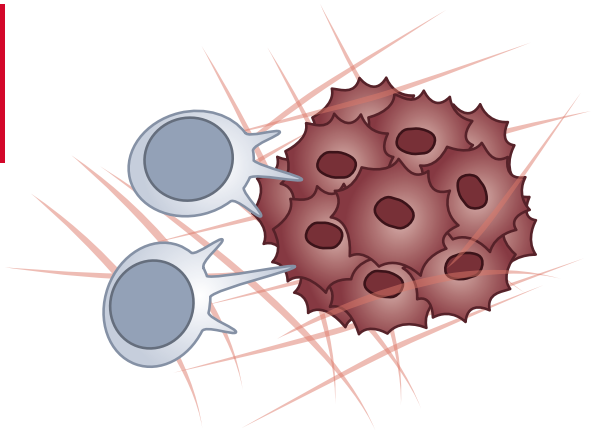
Tube formation assay to test for angiogenesis in ovarian cancer in the µ-Plate 96 Well 3D

Noh, K., et al. (2020). The hidden role of paxillin: localization to nucleus promotes tumor angiogenesis. Oncogene. 10.1038/s41388-020-01517-3
Read article

Tube formation assay to analyze angiogenesis in lung cancer using the µ-Slide 15 Well 3D

Xu, Z., et al. (2021). Endothelial deletion of SHP2 suppresses tumor angiogenesis and promotes vascular normalization. Nature Communications. 10.1038/s41467-021-26697-8
Read article

Tumor Microenvironment and Immunooncology

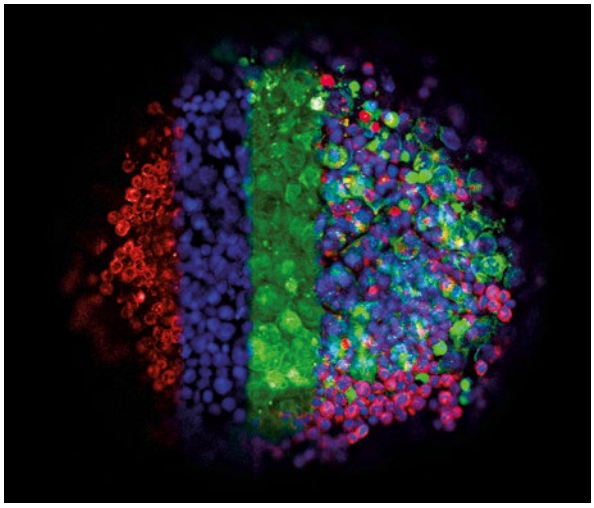


Solid tumors are surrounded and interact with the **tumor microenvironment (TME)**, which is composed of extracellular matrix, fibroblasts, immune cells, lymphatic and vascular endothelial cells, and further stroma cells. Contrasting the classical tumor cell-oriented basic research, the TME has gained more and more attention in recent decades, opening new perspectives for therapeutic approaches (Balkwill *et al.*, 2012).

Many immune cell types, such as T cells and myeloid cells (e.g., tumor-associated macrophages, TAMs), are part of the TME and have already been intensely investigated for their therapeutic suitability. Depending on the cell subtype, they can have both pro- or anti-tumor activity and be associated with a good or a bad prognosis. Especially T cells harbor the potential to be used to destroy tumor cells in a patient-specific approach: T cells with engineered chimeric antigen receptors (CAR T cells) have been developed for **adoptive T cell transfer therapy** and showed clinical success for the treatment of B cell lymphomas.

In a healthy system, the immune checkpoints limit the immune response to prevent collateral tissue damage. However, in a tumorigenic environment, cancer cells can control these checkpoints to inhibit their own destruction by the immune system. The clinical approval of **immunotherapy** of specific cancer types, such as melanoma, by blocking immune checkpoints (e.g., by anti-CTLA-4, anti-PD-1, or anti PD-L1), created an entirely new approach of tumor treatment, which has improved the outcome of thousands of patients. However, not all of them profit from the immunotherapy, with some not responding to the treatment and others gaining resistance. These cases imply that more basic research is still needed to evaluate a more patient-specific treatment strategy (Topalian *et al.*, 2015; Waldman *et al.*, 2020).

Various *in vitro* approaches exist for deciphering the communication and interaction mechanisms between tumor cells and their microenvironment: **CAR-T cell killing assays, co-culture of cancer cells and immune cells** (or other stromal cells), and **rolling and adhesion assays**, just to name a few, help researchers worldwide to gain knowledge of this important field of cancer research.



This image shows the three-dimensional growth of germ cell tumor cells (green, GFP) and immune cells (lymphocytes, red, mCherry) using the hanging drop technique. Cell nuclei appear in blue (DAPI). In 3D culture, a close interaction between the diverse cell types develops. The drops were transferred into an ibidi µ-Plate 96 Well. The image was acquired using a Zeiss LSM 710 confocal microscope with a 10x objective. Data by Gillian Ludwig, Daniel Nettersheim, Translational Urooncology, Department of Urology, University Hospital Düsseldorf, Germany.

Balkwill, F.R., et al. (2012). The tumor microenvironment at a glance. Journal of Cell Science. 10.1242/JCS.116392
Read article

Topalian, S.L., et al. (2015). Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell. 10.1016/J.CCELL.2015.03.001
Read article

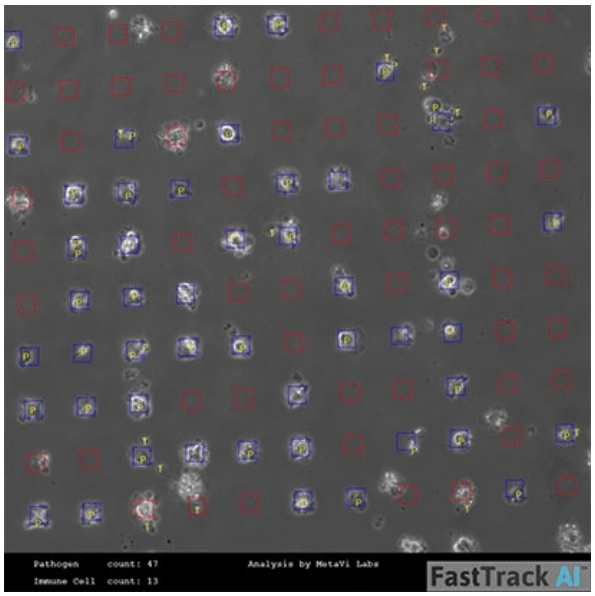
Waldman, A.D., et al. (2020). A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nature Reviews Immunology. 10.1038/s41577-020-0306-5
Read article

Hypoxia is common in cancer cells and the TME as a result of excessive tumor growth and insufficient vessel connection and oxygen supply. It can influence cancer biology in many ways, promotes angiogenesis, and correlates with cancer progression and a poor prognosis. Therefore, when working with cancer cells or stromal cells, researchers should take working under hypoxic conditions under consideration, as it reflects the physiological oxygen levels in tumors more accurately. With the ibidi Stage Top Incubators, hypoxic conditions can be mimicked on every inverted standard microscope during live cell imaging experiments.

Experimental Example: Automated Analysis of CAR-T Cell Killing Assays

CAR-T cells represent a promising new cancer therapy tool. Live cell imaging allows to analyze T cell/cancer cell interaction in real time with single cell resolution. However, analysis of confluent cell layers is very time-consuming and therefore not possible in high throughput screens. To facilitate high throughput label-free analysis of T cell potency in a live cell imaging setup, we generated arrays of homogenously distributed cancer cells using the ibidi Micropatterning technology. By combining optical analysis and advanced image processing, cytotoxic T cell activity over time on a single cell level can be evaluated without the use of any labeling.

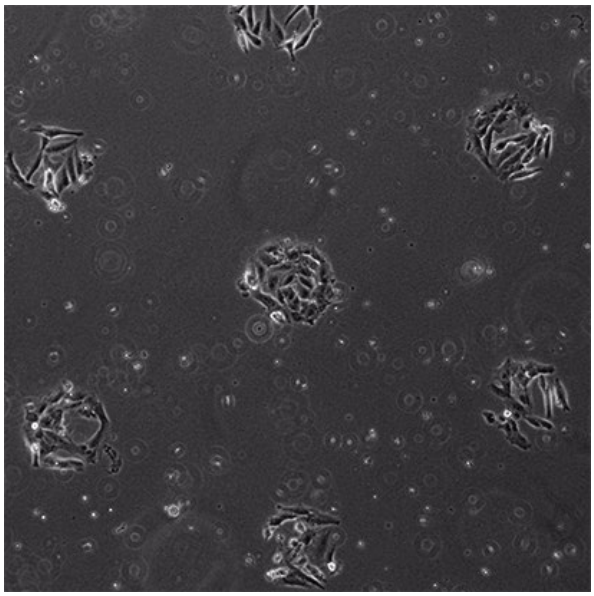
Single Cells in a 2D Environment



Time lapse microscopy of a CAR-T cell killing assay with RCC-26 renal tumor cells and JB4 T cells on a single cell pattern. (µ-Slide VI$^{0.4}$, 20 µm squares, 120 µm distance, rectangular). Data were analyzed using FastTrack AI by MetaVi Labs.

Watch the movie here.

Multi-Cell Spots in a 3D Collagen Matrix



RCC-26 renal cancer cells immobilized on multi-cell pads (µ-Slide VI$^{0.4}$, 200 µm circles, 600 µm distance, hexagonal). Effector T cells applied in a collagen I matrix (Collagen Type I, Rat Tail) induce apoptotic body formation of cancer cells.

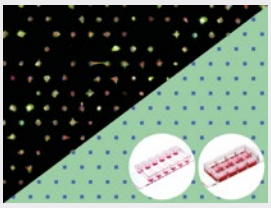
Watch the movie here.

ibidi Solutions for Immunooncology and Analysis of Tumor Microenvironment

The ibidi µ-Slides and µ-Dishes include different geometries that combine optimal conditions for functional cell-based assays using cancer cells, immune cells, or other stromal cell types. They are ideal for immunofluorescence, live cell imaging, and high-resolution microscopy. The ibidi labware is available with the ibidi Polymer Coverslip and the ibidi Glass Coverslip. For high-throughput experiments, we offer the ibidi µ-Plates, which are available with 24 or 96 wells.

The ibidi Stage Top Incubators provide physiological conditions for live cell imaging on every standard inverted microscope. They include CO_2 and O_2 control (e.g., for hypoxia experiments) as well as actively controlled humidity. They are available for single slides and dishes as well as for multiwell plates.

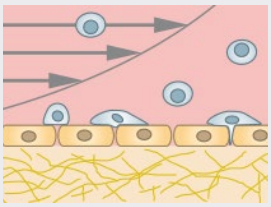
µ-Slides With Single-Cell µ-Pattern are ready-to-use micropatterned slides with ideal spacing for single cell assays. The µ-Slides With Multi-Cell µ-Pattern enable spatially defined cell adhesion for spheroid and organoid generation. Both solutions have been optimized for long-term culture and high-resolution imaging and are useable for versatile cancer cell/immune cell interaction studies (e.g., a CAR-T cell activity assay) and much more.



The ibidi Pump System is ideal for long-term cell culture under flow with defined shear stress values and is compatible with all µ-Slides with Luer adapters. It simulates defined continuous and pulsatile laminar flow, and oscillatory flow to study cells in a more physiological environment. It is optimal for rolling and adhesion assays, transmigration, and invasion studies. Also, cells, spheroids, and organoids can be perfused for optimal nutrition.

ibidi provides a variety of Channel Slides with different geometries. The µ-Slide I Luer family has one channel designed for standard flow experiments and rolling and adhesion assays. The µ-Slide VI has 6 channels ideal for parallel flow assays. Both are available with the ibidi Polymer Coverslip and the ibidi Glass Coverslip, plus different heights and coatings.

The µ-Slide I Luer 3D allows for creating an endothelial barrier without the need for an artificial filter membrane. Endothelial cells can be seeded on a suitable gel matrix, such as Collagen Type I. After connecting the slide to a pump and applying defined shear stress, an *in vivo*-like endothelial barrier is created, which is useful for rolling and adhesion assays or transendothelial migration studies.



The µ-Slide Chemotaxis and the sticky-Slide Chemotaxis are ideal for analyzing single cell migration of immune cells and cancer cells in 2D and 3D, because they quickly create a stable chemotactic gradient. These gradients are easily established in 2D or water-based 3D gels, such as Collagen I gels and Matrigel®, because the gel structure does not hinder the formation of a soluble gradient by diffusion.

Selected References

3D chemotaxis assays of CD8⁺ peripheral blood T cells (PBTs) from patients with head and neck squamous cell carcinoma (HNSCC) using the µ-Slide Chemotaxis

Newton, H.S., et al. L. (2020). PD1 blockade enhances K^+ channel activity, Ca^{2+} signaling, and migratory ability in cytotoxic T lymphocytes of patients with head and neck cancer. Journal for Immunotherapy of Cancer. 10.1136/JITC-2020-000844
Read article

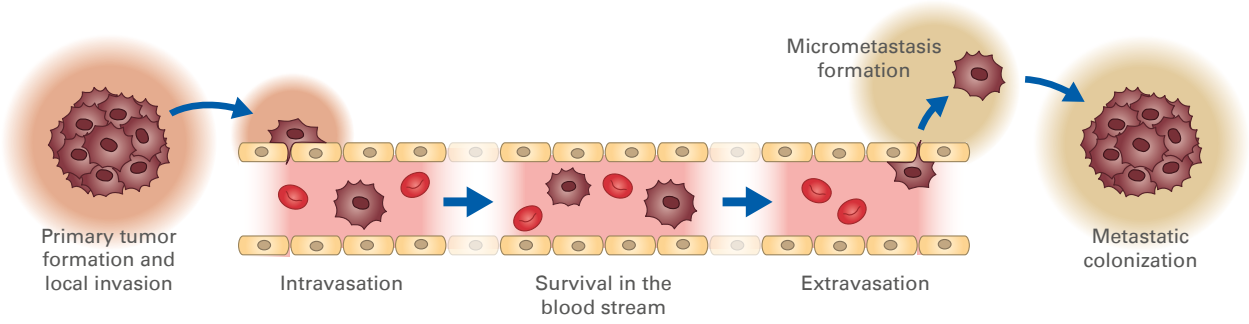
Co-culture assay with tumor cells and macrophages in a breast cancer model using the µ-Dish 35 mm, high

Sharma, V.P., et al. (2021). Live tumor imaging shows macrophage induction and TMEM-mediated enrichment of cancer stem cells during metastatic dissemination. Nature Communications. 10.1038/s41467-021-27308-2
Read article

Co-culture assay with bone marrow-derived macrophages (BMDM) and tumor cells in the µ-Plate 96 Well Black

Luthria, G., et al. (2020). In vivo microscopy reveals macrophage polarization locally promotes coherent micro-tubule dynamics in migrating cancer cells. Nature Communications. 10.1038/s41467-020-17147-y
Read article

Invasion and Metastasis



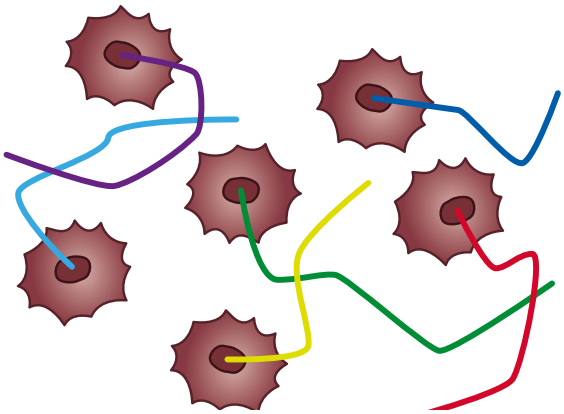
Metastasis is responsible for more than 90% of cancer-related deaths. The invasion-metastasis cascade is a multi-step process, which involves the invasion of epithelial cells from the primary tumor into the adjacent stroma and through the extracellular matrix (ECM), intravasation of cancer cells into the bloodstream, their dissemination to distant sites, the formation of micrometastases, and finally the establishment of secondary tumors in distant organs.

Each of these complex events is driven by the deregulated molecular pathways that operate within tumor cells. Also, the cell-nonautonomous interactions between cancer cells and non-neoplastic stromal cells play a crucial role during the invasion-metastasis cascade. Altogether, this finally generates macroscopic, clinically detectable metastases with life-threatening abilities.

Breaking down the metastatic cascade into single steps and analyzing the different sub-processes, such as chemo-taxis, (transendothelial) cell migration, cell behavior in the blood stream (cancer cells under shear stress), and rolling/adhesion, provides insights into the mechanisms of invasion and metastasis. These novel discoveries will bring progress to metastasis research, finally leading to novel basic research findings and their translation into the clinic.

Valastyan, S., & Weinberg, R. A. (2011). Tumor metastasis: molecular insights and evolving paradigms. Cell. 10.1016/J.CELL.2011.09.024
Read article

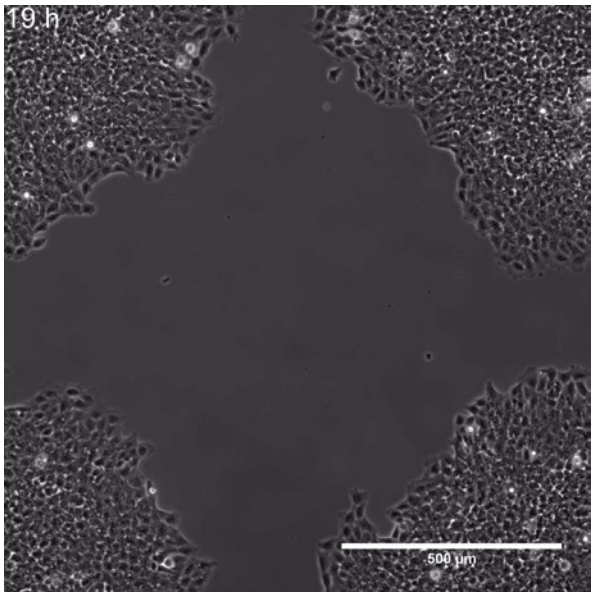
Tumor Cell Migration



Cell migration occurs during the whole cascade of cancer development and is especially important during invasion, which is the initial step of metastasis. Following chemotactic migration, a cancer cell invades into the surrounding tissue and the local vasculature. This step is guided by protrusion (extension) of the cell membrane and its connection to the extracellular matrix. Migration is a precisely regulated process that can be guided by both cell-internal signals and cues of the local microenvironment. Many factors such as mechanical and chemical signals are sensed by specific cellular receptors (e.g., integrins, chemokine receptors, and growth factor receptors), thereby influencing cancer cell migration.

To develop therapies that prevent tumor cell migration, it is necessary to understand the mechanisms of the stimuli in tumor cells and their surrounding microenvironmental cells that lead to migration. Cell migration assays, such as the classical scratch assay or wound healing assay, give information about the speed and characteristics of cell migration (e.g., after a gene knock-down or a drug treatment). If the directed cell migration towards a chemoattractant needs to be measured, a chemotaxis assay should be conducted.

Polacheck, W.J., et al. Tumor cell migration in complex micro-environments. Cellular and Molecular Life Sciences. 10.1007/S00018-012-1115-1
Read article

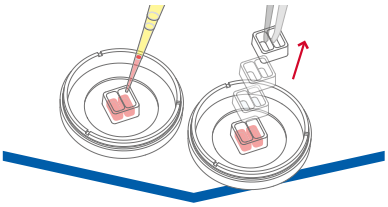


Live cell imaging of a wound healing and migration assay using the MCF7 breast cancer cell line in the Culture-Insert 4 Well. Physiological conditions were maintained with the ibidi Stage Top Incubation System. Objective lens 10x, phase contrast microscopy.

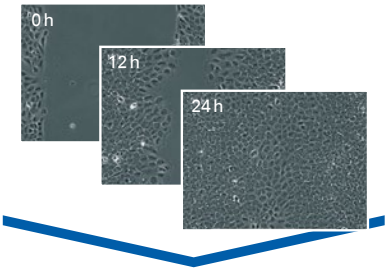
Download a detailed Application Guide at:
ibidi.com/WoundHealingGuide

ibidi Solutions for Tumor Cell Migration Assays

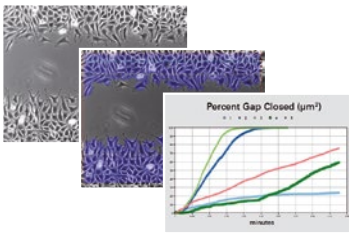
Sample Preparation



Live Cell Imaging



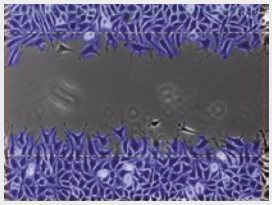
Data Analysis



The ibidi Culture-Inserts with 2 Wells, 3 Wells, or 4 Wells are silicone inserts with a defined cell-free gap for wound healing, migration, 2D invasion assays, and co-cultivation of cells. They are available as individual inserts in a µ-Dish or as 25 pieces for self-insertion. For high-throughput experiments, we provide the Culture-Insert 2 Well 24.

The ibidi Stage Top Incubators provide physiological conditions for live cell imaging on every standard inverted microscope and are ideal for cell migration assays over longer periods of time. They include CO_2 and O_2 control as well as actively controlled humidity. They can be used with single dishes (e.g., the Culture-Insert 2 Well in µ-Dish 35 mm, high) as well as with multiwell plates (e.g., the Culture-Insert 2 Well 24).

Wound Healing FastTrack AI Image Analysis is an AI-based automated image analysis solution for wound healing and cell migration assays, which provides objective and reproducible results within minutes.



Selected References

Analysis of the migration of pancreatic tumor cells using the using the ibidi Culture-Inserts

Cheng, Y., et al. (2018). Role of metastasis-associated lung adenocarcinoma transcript-1 (MALAT-1) in pancreatic cancer. PLOS ONE. 10.1371/JOURNAL.PONE.0192264
Read article

Analysis of wound healing in a breast cancer model using the ibidi Culture-Inserts

Martinez-Ordoñez, A., et al. (2018). Breast cancer metastasis to liver and lung is facilitated by Pit-1-CXCL12-CXCR4 axis. Oncogene. 10.1038/s41388-017-0036-8
Read article

Migration assay with prostate cancer cells using the ibidi Culture-Inserts

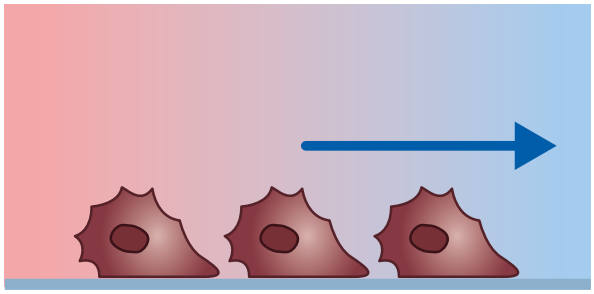
Tseng, J.C., et al. (2020). ROR2 suppresses metastasis of prostate cancer via regulation of miR-199a-5p–PIAS3–AKT2 signaling axis. Cell Death & Disease. 10.1038/s41419-020-2587-9
Read article

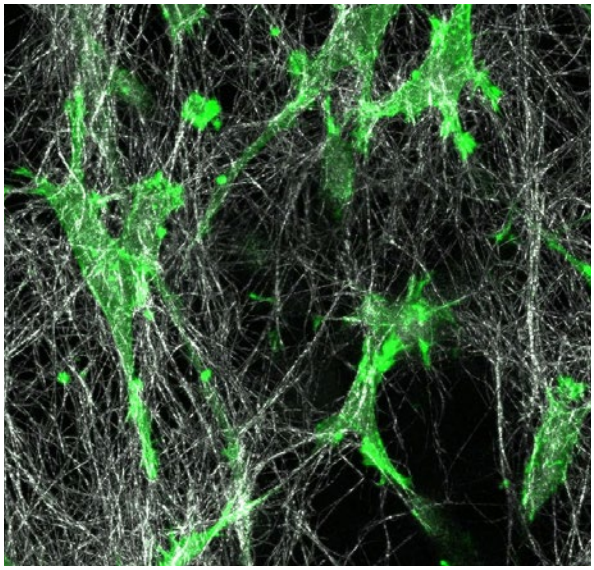
Chemotaxis

Chemotaxis of cancer cells and their associated inflammatory and stromal cells is essential during tumor progression and dissemination. It especially plays a role during the metastatic cascade, where a tumor cell invades, intravasates, extravasates, and finally grows at a distant site. Chemotaxis is tightly regulated in healthy cells and mediated by chemokines, growth factors, and their receptors. However, during cancer progression, reprogramming of the chemotactic pathways can abrogate this regulation in favor of metastasis.

A **chemotaxis assay** is conducted to analyze whether or not a cell type directly orients and migrates towards a defined chemoattractant. Chemotaxis is a critical process during cancer development and immune infiltration, making chemotaxis assays a powerful tool in cancer research.

Roussos, E.T., et al. (2011). Chemotaxis in cancer. Nature Reviews Cancer. 10.1038/NRC3078
Read article



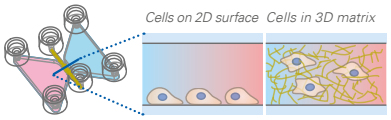


3D live cell imaging of migrating HT-1080 cancer cells in a Collagen matrix. LifeAct-expressing HT-1080 cells (green) were seeded in a 1.5 mg/ml Collagen Type I, Rat Tail layer (white) in the µ-Slide Chemotaxis. Cell migration was documented by taking a photo every 300 seconds on a Zeiss Confocal Microscope LSM 880 AxioObserver using a water immersion objective lens 40x/1.2.

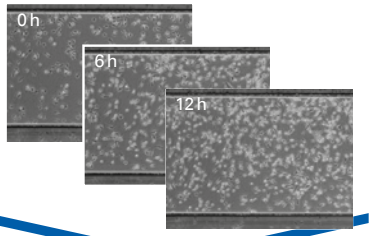
Download a detailed Application Guide at:
ibidi.com/ChemotaxisGuide

ibidi Solutions for Chemotaxis Assays

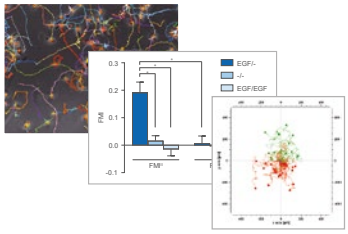
Sample Preparation



Live Cell Imaging



Data Analysis

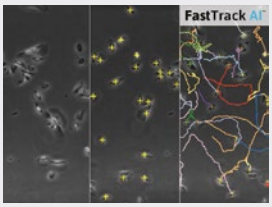


The [µ-Slide Chemotaxis] and the [sticky-Slide Chemotaxis] are ideal for analyzing single cell migration in 2D and 3D.

Chemotactic gradients can be easily established in water-based 3D gels, such as [Collagen I] gels and Matrigel®, because the gel structure does not hinder the formation of a soluble gradient by diffusion.

The [ibidi Stage Top Incubators] provide physiological conditions for live cell imaging during chemotaxis assays on every standard inverted microscope. They include CO_2 and O_2 control as well as actively controlled humidity. They can be used with single slides or with up to 4 slides (e.g., the [µ-Slide Chemotaxis]).

[Chemotaxis FastTrack AI Image Analysis] is an AI-based automated image analysis solution for chemotaxis assays, which provides fast and powerful cell tracking for objective and reproducible analysis. Results are received within minutes without manual tracking.



Selected References

Evaluation of the chemotactic function of NET-DNA (neutrophil extracellular traps) in breast cancer cells using the µ-Slide Chemotaxis

Yang, L., et al. (2020). DNA of neutrophil extracellular traps promotes cancer metastasis via CCDC25. Nature. 10.1038/s41586-020-2394-6
Read article

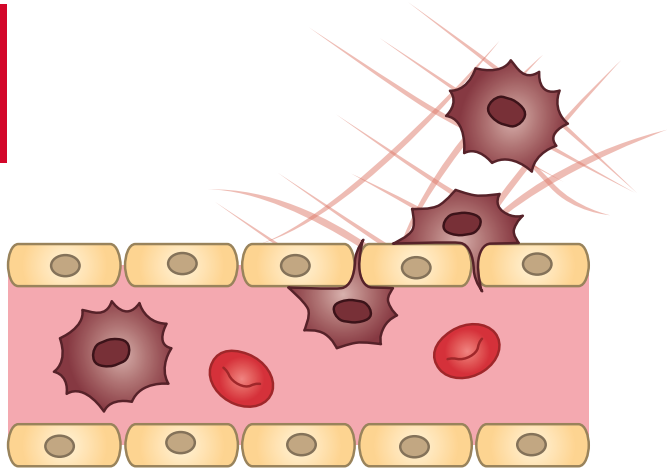
Analysis of the motility of single tumor cells using the µ-Slide Chemotaxis in different cancer types

Agarwal, E., et al. (2021). A cancer ubiquitome landscape identifies metabolic reprogramming as target of Parkin tumor suppression. Science Advances. 10.1126/sciadv.abg7287
Read article

Directed migration in cancer-associated fibroblasts (CAFs) and mesenchymal stem cells using the µ-Slide Chemotaxis

Mestre-Farrera, A., et al. (2021). Glutamine-Directed Migration of Cancer-Activated Fibroblasts Facilitates Epithelial Tumor Invasion. Cancer Research. 10.1158/0008-5472.CAN-20-0622
Read article

Cancer Cells Under Shear Stress



Cancer cells with high metastatic potential can pass through the endothelial cell barrier of a blood or lymphatic vessel (**intravasation**). This **transendothelial migration** allows them to enter the blood stream and be transported to distant locations in the body. Once in the blood flow, these circulating tumor cells (CTCs) are exposed to new environmental conditions, such as **shear stress**. These conditions change the cell's gene expression patterns, altering cell behavior and morphology.

Extravasation of CTCs at a distant site, which is a critical step for metastasis, is initialized by the **rolling and adhesion** of these cancer cells to the endothelium. Then, the cells transmigrate once again through the endothelium, where they start proliferating to form a secondary tumor.

Flow assays are conducted to understand the altered cancer cell behavior under shear stress. Analysis of the factors that guide intravasation, transendothelial migration, rolling and adhesion, and extravasation will give more insight into the mechanisms that underlie the metastatic cascade and open new possibilities for therapeutic targets.

Download a detailed Application Guide at: ibidi.com/FlowGuide

Selected References

Microfluidic experiments mimicking tumor blood vessels using the ibidi Pump System and µ-Slide I$^{0.2}$ Luer with HUVECs

John, A., et al. (2020). Urothelial Carcinoma of the Bladder Induces Endothelial Cell Activation and Hypercoagulation. Molecular Cancer Research. 10.1158/1541-7786.MCR-19-1041
Read article

Assembling of a blood brain barrier (BBB) *in vitro* system using the ibidi Pump System to check nanoparticles (for treatment of glioblastoma multiforme) for their ability to cross the barrier

Grillone, A., et al. (2019). Nutlin-loaded magnetic solid lipid nanoparticles for targeted glioblastoma treatment. Nanomedicine. 10.2217/nnm-2018-0436
Read article

Measurement of the attachment of glioblastoma and breast cancer cells to endothelial cells under physiological blood flow using the ibidi Pump System and the µ-Slide I$^{0.6}$ Luer

Nguemgo Kouam, P., et al. (2019). The increased adhesion of tumor cells to endothelial cells after irradiation can be reduced by FAK-inhibition. Radiation Oncology. 10.1186/s13014-019-1230-3
Read article

ibidi Solutions for Shear Stress Assays

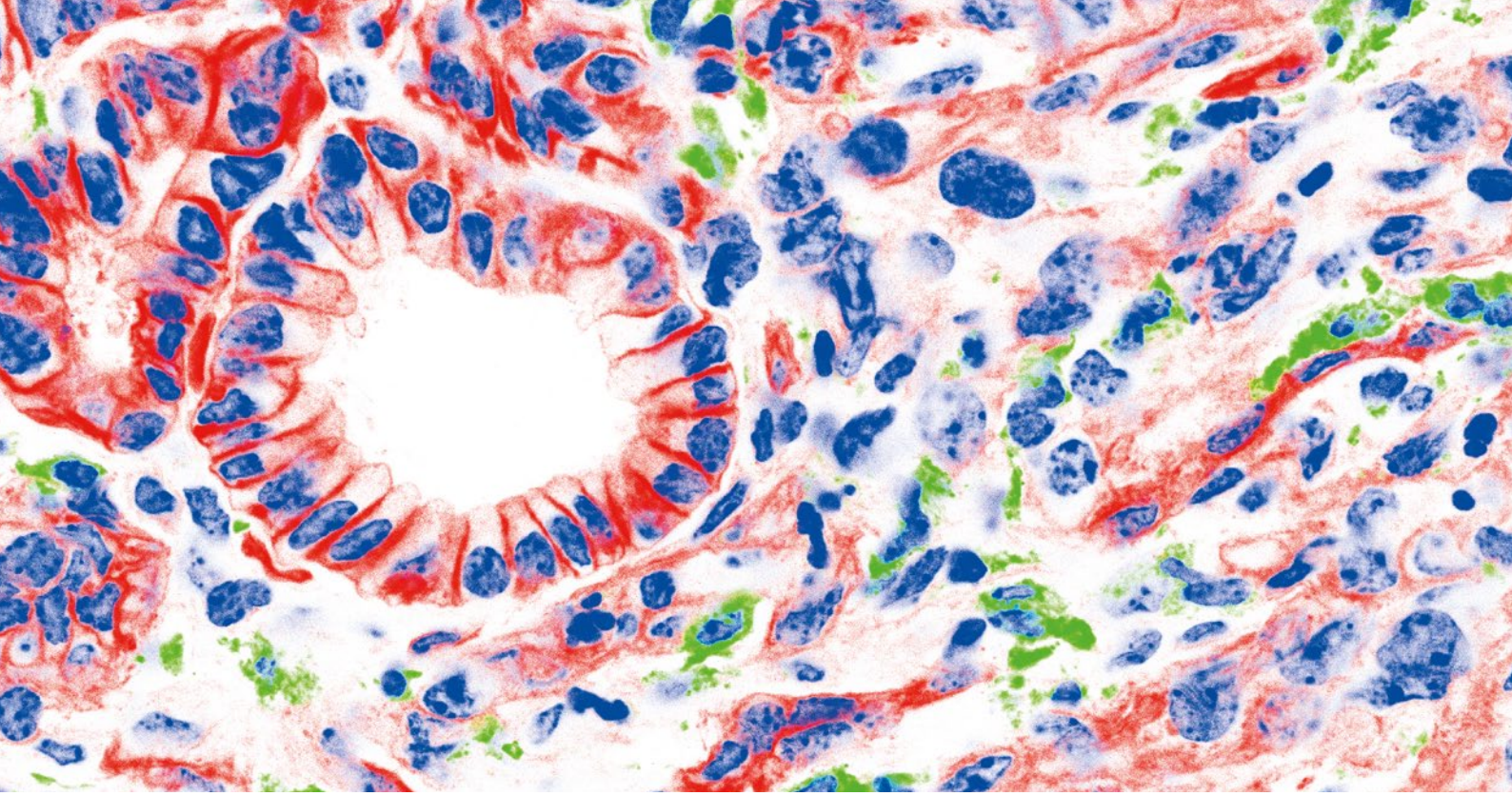
The ibidi Pump System is ideal for long-term cell culture under flow with defined shear stress values and is compatible with all µ-Slides with Luer adapters. It simulates defined continuous and pulsatile laminar flow, and oscillatory flow to study cells in a more physiological environment. It is optimal for rolling and adhesion assays, transmigration and invasion studies. Also, cells, spheroids, and organoids can be perfused for optimal nutrition.

ibidi provides a variety of Channel Slides with different geometries. The µ-Slide I Luer family have one channel for standard flow experiments and rolling and adhesion assays. The µ-Slide VI has 6 channels and can be used for parallel flow assays. Both are available with the ibidi Polymer Coverslip and the ibidi Glass Coverslip, plus different heights and coatings.

In the µ-Slide III 3D Perfusion, single cells, spheroids, or organoids can be cultivated in or on a gel layer or embedded in a 3D matrix. The special channel geometry allows for superfusion with a low flow rate, ensuring optimal oxygen and nutrient supply. This setup makes long-term cultivation possible for up to several weeks. Additionally, the thin coverslip bottom allows for high-resolution imaging.

The µ-Slide I Luer 3D allows for creating an endothelial barrier without the need of an artificial filter membrane. Endothelial cells can be seeded on a suitable gel matrix, such as Collagen I. After connecting the slide to a pump and applying defined shear stress, an *in vivo*-like endothelial barrier is created, which is useful for rolling and adhesion assays or transendothelial migration studies.

Endothelial cells can be seeded on a suitable gel matrix, such as Collagen Type I.



ibidi®
cells in focus

Manufacturer / Supplier

ibidi GmbH
Lochhamer Schlag 11
82166 Gräfelfing
Germany

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

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

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

North American Headquarters

ibidi USA, Inc.
2920 Marketplace Drive
Suite 102
Fitchburg, WI 53719
USA

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

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

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

FL_AG_042, V 1.1 2023/06

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

ibidi.com