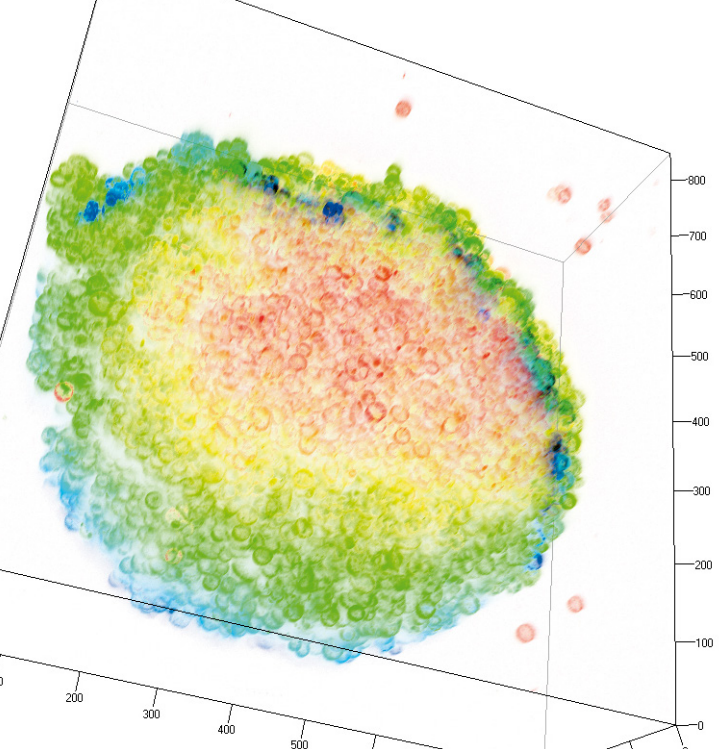


cells in focus

ibidi Application Guide

3D Cell Culture

Selected Publications

P. Xu et al. Modulation of Intestinal Epithelial Permeability by Plasma from Patients with Crohn's Disease in a Three-dimensional Cell Culture Model. Scientific Reports, 2019, 10.1038/s41598-018-38322-8
Read article

E. Hoque Apu, S.U. Akram, J. Rissanen, H. Wan and T. Salo. Desmoglein 3 – Influence on oral carcinoma cell migration and invasion. Experimental Cell Research, 2018, 10.1016/j.yexcr.2018.06.037
Read article

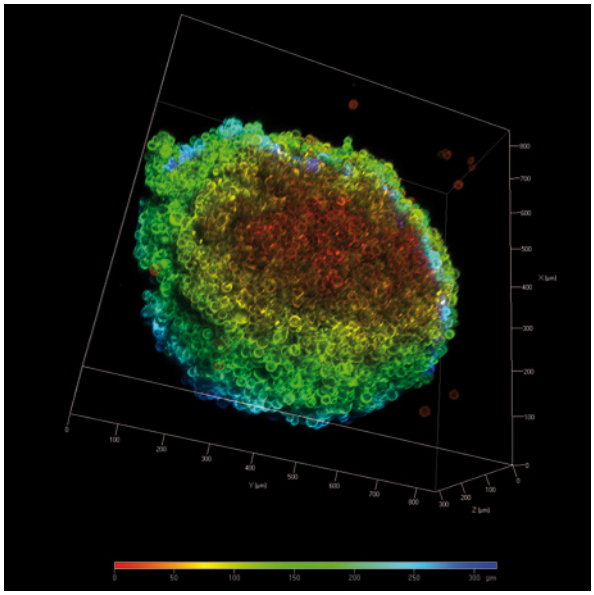
M. Dietrich et al. Guiding 3D cell migration in deformed synthetic hydrogel microstructures. Soft Matter, 2018, 10.1039/C8SM00018B
Read article

H. Grobe, A. Wüstenhagen, C. Baarlink, R. Grosse and K. Grikscheit. A Rac1-FMNL2 signaling module affects cell-cell contact formation independent of Cdc42 and membrane protrusions. PloS one, 2018, 10.1371/journal.pone.0194716
Read article

3D Cell Culture

The majority of cells in living tissue grow in a three-dimensional microenvironment, where they communicate and interact with each other and their surroundings. Animal cells are embedded in the extracellular matrix (ECM), which is composed of proteoglycans and fibrous proteins (mainly collagen, elastin, and fibronectin). This complex, dynamic, and tissue-specific 3D structure provides physical scaffolding for the cells and initiates cues that influence cell differentiation and behavior.

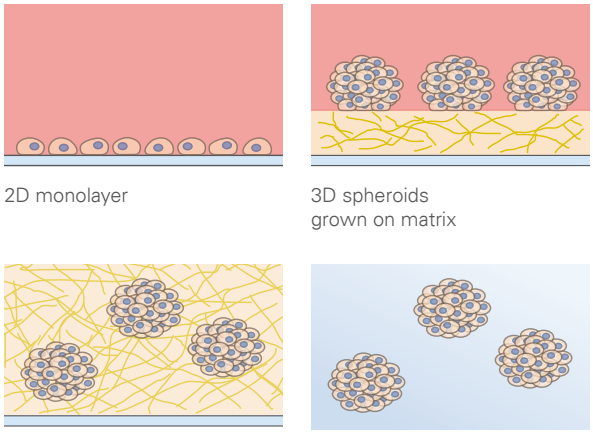
When cultured in a traditional, two-dimensional *in vitro* environment, the cells are attached to a flat surface (e.g., a monolayer in a standard cell culture dish) and can only grow and migrate on the substrate. In a 3D *in vitro* setup, the cells are either grown in suspension on a non-adhesive surface, or they can be embedded in or on a 3D matrix (e.g., Matrigel® or collagen I) that mimics the ECM and allows them to grow in all three directions.



Confocal laser scanning microscopy projection of an HT-1080 LifeAct spheroid. The colors indicate the distance from the surface. Warm colors = close to the surface, cold colors = distant from the surface.

Compared to a 2D environment, cells will behave differently when they are inside a 3D gel matrix. In many cases, a 3D environment reflects the *in vivo* situation more accurately. This should be considered when analyzing cell behavior, differentiation, response to drug treatment, and gene and protein expression.

Not surprisingly, many cell culture approaches have been adapted to a 3D environment, This includes drug screenings that use spheroids and organoids, which are indispensable nowadays as tumor models.



3D cell culture applications in comparison to the traditional 2D monolayer.

Edmondson R, Broglie JJ, Adcock AF, Yang L (2014) Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol 12(4):207–18. 10.1089/adt.2014.573.
Read article

Frantz C, Stewart KM, Weaver VM (2010) The extracellular matrix at a glance. J Cell Sci 123(Pt 24):4195–200. 10.1242/jcs.023820.
Read article

Matrices for 3D Cell Culture

A variety of matrices are currently being used for culturing cells in a 3D environment, including a wide range of natural proteins to synthetic scaffolds. The choice of a suitable matrix strongly depends on the cell type being used and specific experimental questions. Hydrogels, such as collagen and fibrin, are water-swollen polymer networks that are commonly used. They have the ability to substitute the natural matrix in 3D cell culture experiments, because they mimic several key features of the native extracellular matrix (ECM).

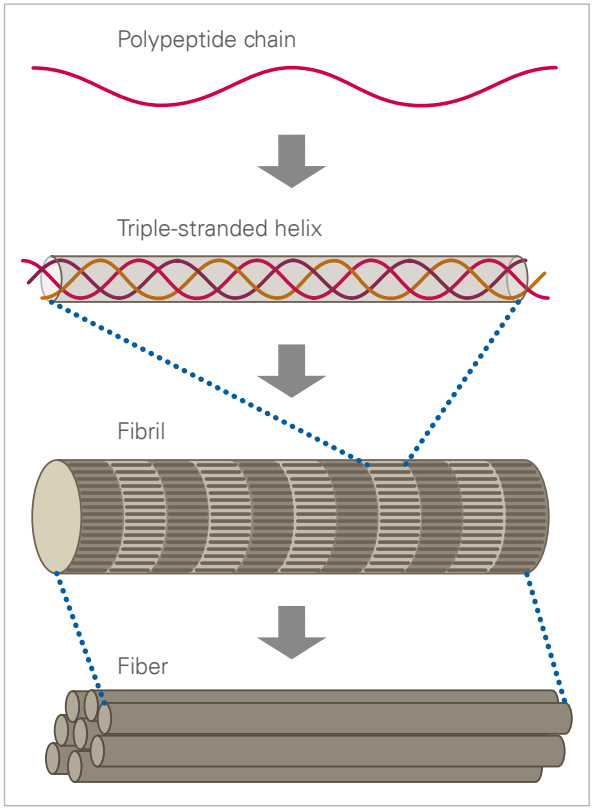
Collagen I is the main component of connective tissue and is abundant in the mammalian body. The fibrous protein consists of three α-chains that combine to create a rope-like triple helix, thus providing tensile strength to the ECM. The triple helices aggregate and form fibrils in a self-organized manner. *In vivo*, the fibrils polymerize into fibers to form tissue such as tendon or dermis.

Collagen (mostly type I) is widely used in 3D cell culture for modeling the extracellular matrix. To embed the cells in the matrix, they are mixed with the liquid gel and pipetted into the vessel. Raising the pH and temperature leads to self-assembly of the collagen fibrils, which results in gelation and the encapsulation of the cells.

Matrigel® is a collagen- and laminin-containing hydrogel that is used in many 3D cell culture approaches (e.g., organoid culture). It is derived from Engelbreth–Holm–Swarm (EHS) mouse sarcoma tumors and consists mainly of laminin, type IV collagen, and entactin.

Detailed comparison of hydrogels, including their applications, advantages, and disadvantages:

Caliari SR, Burdick JA (2016) A practical guide to hydrogels for cell culture. Nat Methods 13(5):405–414. 10.1038/nmeth.3839
Read article



ibidi Solution

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.

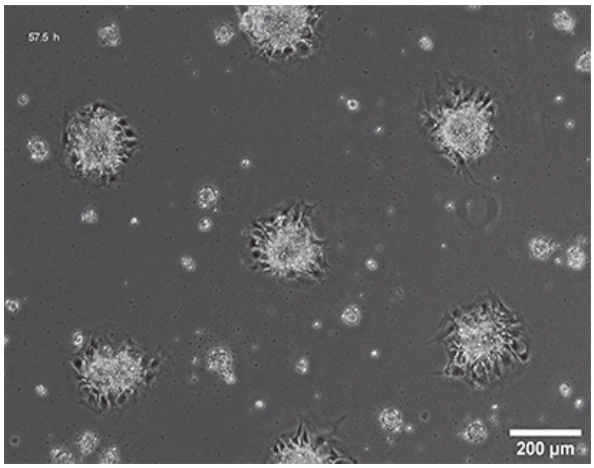
Read our Application Note about how to prepare a 3D gel using the ibidi Collagen Type I, Rat Tail here: AN 26: Collagen I Gel for 3D Cell Culture.

Spheroid and Organoid Culture

Spheroids are cells that adhere to each other under three-dimensional, non-adherent culture conditions. They lack stem cells, which means that they consist of fully differentiated cells. They can be generated by placing them into a scaffold-free suspension using the hanging drop or forced floating method, for example.

Spheroids are not capable of self-renewal and further differentiation. An exception are tumor cell spheroids, because due to the unlimited proliferation capacities of the tumor cells, they are able to divide and renew. Therefore, spheroids are a useful model for examining tumor cell behavior, such as for large-scale drug screenings.

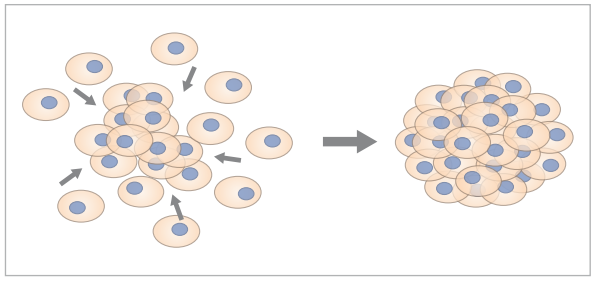
Read here to see a detailed protocol for spheroid generation in the µ-Plate Angiogenesis: AN 32: Generation of Spheroids (PDF)



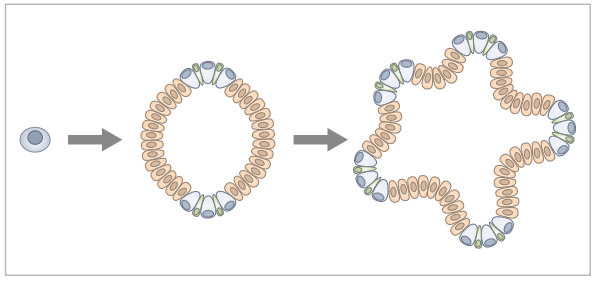
NIH-3T3 cells forming defined spheroids on the ibidi µ-Pattern. Cells were seeded on 200 µm adhesion spots in a µ-Slide VI$^{0.4}$ and kept under flow (3 dyn/cm²) for 14 days.

Organoids are cultured "mini organs". They can be generated from adult stem cells (ASCs) or pluripotent stem cells (PSCs). When cultured in a three-dimensional matrix/scaffold (e.g., Matrigel® or collagen), these cells differentiate into organ-specific cell types that build small functional organs.

The first generation of intestinal organoids, which were created from an Lgr5^{+} stem cell by Sato et al., initiated the establishment of many protocols for organoid generation from different organs, such as intestine, liver, brain, prostate, kidney, pancreas, lung, and thyroid. Importantly, they can be edited using technologies such as CRISPR, making them a powerful tool for studies about personal therapy, organogenesis, and drug screening.



Spheroids are cell aggregates, which are often generated from cancer cells.



Organoids are cultured miniature versions of organs, which are derived from stem cells.

Sato T, et al. (2009) Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature 459(7244):262–265. 10.1038/nature07935.
Read article

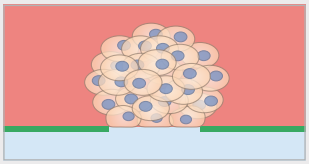
Drost J, Clevers H (2018) Organoids in cancer research. Nat Rev Cancer 18:407–418. 10.1038/s41568-018-0007-6.
Read article

Tuveson D, Clevers H (2019) Cancer modeling meets human organoid technology. Science 364(6444):952–955. 10.1126/science.aaw6985.
Read article

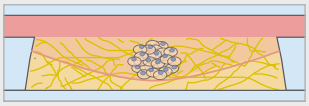
ibidi Solutions

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. Read our Application Note about how to prepare a 3D gel using the ibidi Collagen Type I, Rat Tail here: AN 26: Collagen I Gel for 3D Cell Culture.

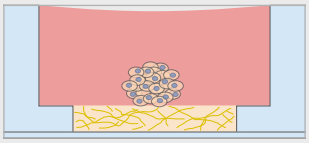
Bioinert is a stable, biologically inert surface for long-term culture and high-resolution microscopy of spheroids, organoids, and suspension cells on a non-adherent surface without any cell or biomolecule adhesion. It is currently available as the µ-Dish 35 mm, high Bioinert.



The ibidi µ-Patterning technology enables spatially defined cell adhesion for spheroid and organoid generation, long-term culture, and high resolution imaging. Defined adhesion spots are able to catch all adherent single cells from a cell suspension. The surrounding Bioinert 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.



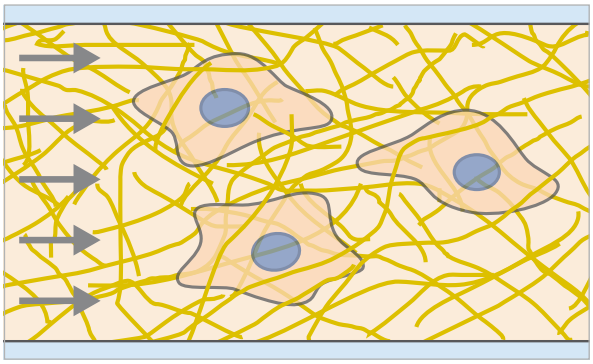
In the µ-Slide III 3D Perfusion, spheroids or organoids can be cultivated in or on a gel layer or embedded in a 3D matrix. The special channel geometry allows for superfusion with a low flow rate (e.g., when utilizing the ibidi Pump System). This setup makes long-term cultivation possible for up to several weeks. Additionally, the thin coverslip bottom allows for high-resolution imaging.



The µ-Slide Angiogenesis or the µ-Plate Angiogenesis 96 Well are easy, cost-effective solutions for the 3D cultivation and microscopy of spheroids and organoids on/in gel matrices. The gel layer is directly connected to the medium reservoir above, which allows for fast and easy medium exchange by diffusion.

3D Cell Culture Under Interstitial Flow

In vivo, many cell types are constantly exposed to shear stress. When culturing them in an *in vitro* 3D matrix, a soft interstitial flow can be applied by perfusing them with growth medium or any reagent or drug of choice. By doing this, conditions close to the cells' natural environment can be established.



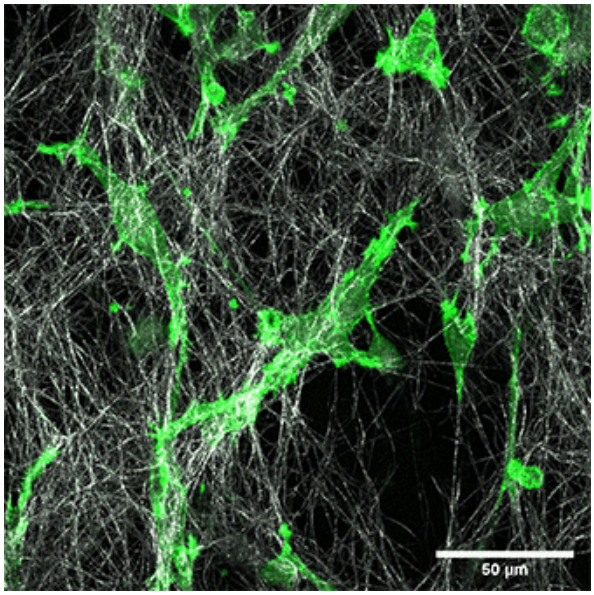
ibidi Solutions

The ibidi Channel Slides, including the µ-Slide III 3D Perfusion, the µ-Slide I Luer and the µ-Slide VI families, allow for the seeding of the cells in a 3D matrix and the application of an interstitial flow (e.g., using the ibidi Pump System).

Single Cells in a 3D Matrix

In many cases, a 3D environment more closely resembles an *in vivo* situation than a 2D cell culture. Single cells can be cultured and imaged in a 3D gel in order to analyze diverse biological questions, such as cell deformation, migration, tube formation, or ECM degradation. In addition to cultures with only one cell type, the invasion behavior of different cell types (e.g., cancer cells and fibroblasts) can also be investigated in a co-culture experiment.

In order to isolate cells from the gel matrix, the matrix can be degraded enzymatically (e.g., collagen by collagenase). After this, the cells can be either expanded in a new gel matrix or further processed for DNA, RNA, or protein isolation.



LifeAct-expressing HT-1080 cells (green) in a Collagen Type I, Rat Tail layer in the µ-Slide Chemotaxis.

ibidi Solutions

The ibidi Collagen Type I, Rat Tail is a non-pepsinized, native collagen for modeling biological ECM in gel matrices. Its fast polymerization facilitates optimal cell distribution in 3D gels. Read our Application Note about how to prepare a 3D gel using the ibidi Collagen Type I, Rat Tail here: AN 26: Collagen I Gel for 3D Cell Culture.

In the µ-Slide III 3D Perfusion, single cells are embedded in a 3D matrix. The special channel geometry allows for superfusion with a low flow rate (e.g., when utilizing the ibidi Pump System). Unlike in static cultures, the superfusion ensures an 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 Angiogenesis or the µ-Plate Angiogenesis 96 Well allow for easy, cost-effective cultivation and microscopy of single cells and co-cultures on or in 3D gels. The gel layer is directly connected to the medium reservoir above, which allows for fast and easy medium exchange by diffusion.

Chemotaxis and Migration Assays in 3D

A chemotaxis assay is used to analyze directed cell migration towards a chemoattractant. Culturing cells in a 2D environment during a chemotaxis assay might not reflect the *in vivo* situation and therefore result in an altered cell behavior and migration. To overcome this issue, cells can be embedded in a 3D matrix that mimics their natural environment, such as collagen, Matrigel®, or other hydrogels.

Advantages of 3D Chemotaxis Assays

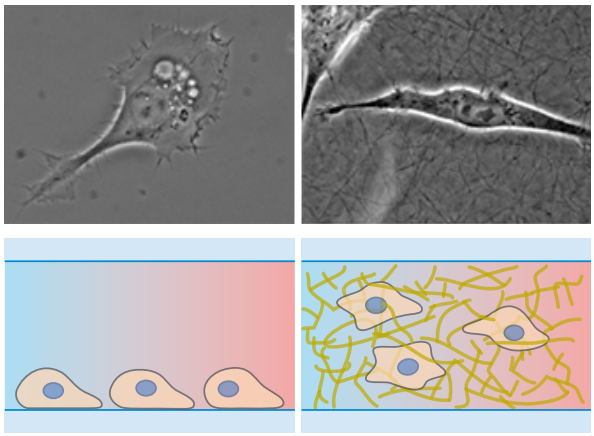
- More *in vivo*-like setting for most cell types
- Highly defined environment (e.g., fibers or matrix)
- Chemotaxis assays with suspension cells possible

Limitations of 3D Chemotaxis Assays

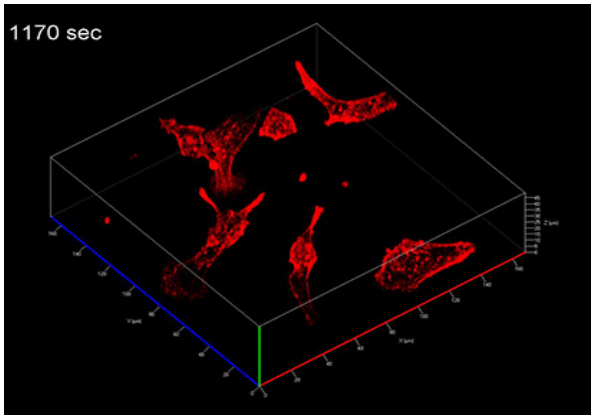
- Difficult gel handling; more parameters to control during the experiment
- Cells might attach to 2D surface, thus creating 2.5D conditions
- Cells might go out of focus during 3D tracking

Find more information about 2D and 3D chemotaxis assays in the following Application Notes:

- AN 17: Chemotaxis 2D and 3D (PDF)
- AN 23: 3D Chemotaxis Protocol for Non-Adherent Cells in a Gel Matrix (PDF)
- AN 24: Chemotaxis of HT-1080 Cells in 2D and 3D (PDF)
- AN 26: Collagen I Gel for 3D Cell Culture (PDF)
- AN 34: Chemotaxis of HUVEC Cells in 2D and 3D (PDF)



Microscopy and schematic of adherent HT-1080 cancer cells on a 2D surface (left), and embedded into a 3D Collagen I gel (right) in the µ-Slide Chemotaxis.



Spinning disk confocal time-lapse microscopy of LifeAct TagRFP transfected HT-1080 cancer cells, which are migrating in a 3D Collagen matrix in the µ-Slide Chemotaxis, 63x oil immersion.

Biswenger V, et al. Characterization of EGF-guided MDA-MB-231 cell chemotaxis in vitro using a physiological and highly sensitive assay system. PLoS One, 2018, 10.1371/journal.pone.0203040.
Read article

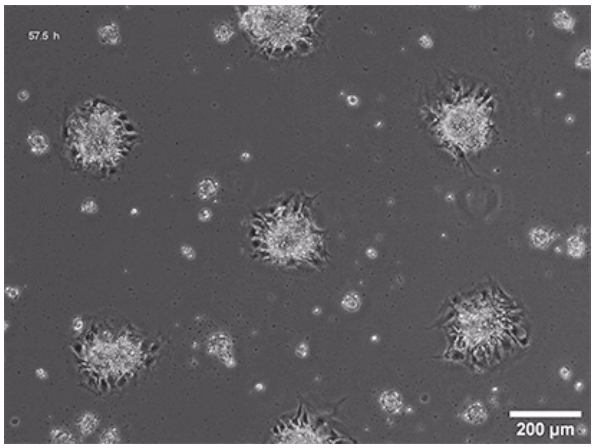
ibidi Solutions

The µ-Slide Chemotaxis and the sticky-Slide Chemotaxis are ideally suited for both 2D and 3D experiments. 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.

Experimental Examples

Spheroid Formation on a Defined Micropattern

The ibidi µ-Patterning technology enables spatially defined cell adhesion for various 2D and 3D cell culture applications. Defined adhesion spots, surrounded by Bioinert, are able to catch all adherent single cells from a cell suspension. Bioinert 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. Here, a suspension of NIH-3T3 cells was seeded on 200 µm adhesion spots. Spheroid generation was documented for 64 hours using phase contrast live cell imaging with a 4x objective lens.



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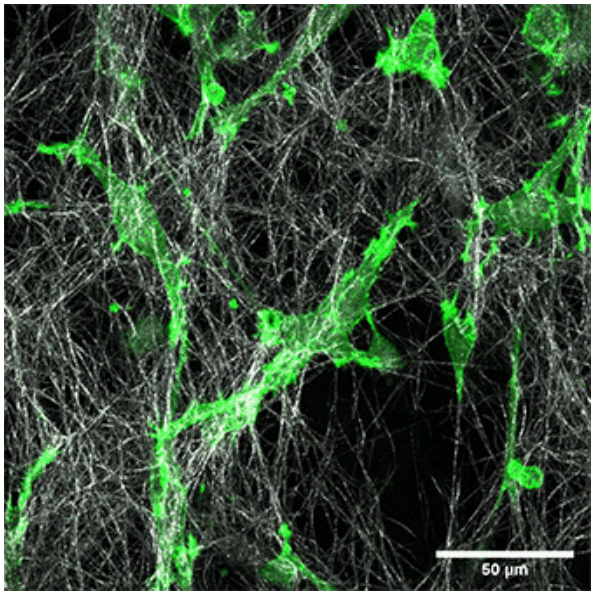
3D Live Cell Imaging of Migrating HT-1080 Cancer Cells in a Collagen Matrix

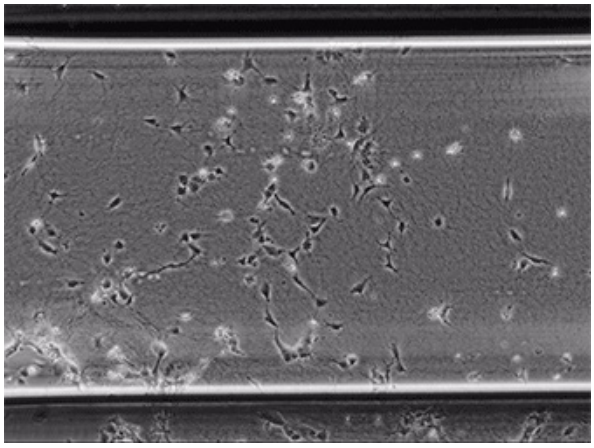
LifeAct-expressing HT-1080 cells (green) were seeded in a 1.5 mg/ml Collagen Type I, Rat Tail layer (white) in the µ-Slide Chemotaxis. Cell migration was documented by taking a photo every 300 seconds on a Zeiss Confocal Microscope LSM 880 AxioObserver using a water immersion objective lens 40x/1.2.



Click here to watch the movie on our website.

Chemotaxis of Endothelial Cells in a 3D Collagen I Gel Towards an FCS Gradient

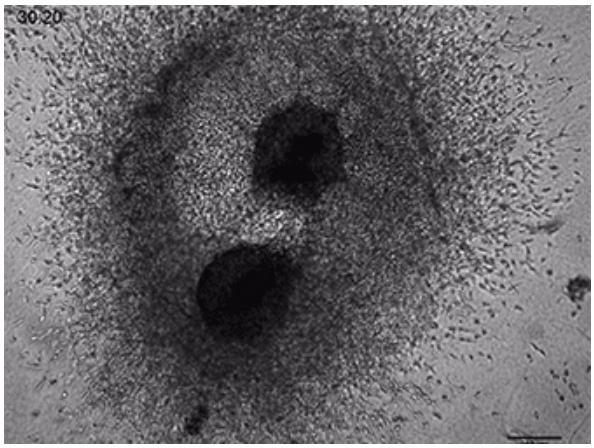
Live cell imaging of Human Umbilical Vein Endothelial Cells (HUVEC) embedded in a 1.5 µg/ml Collagen Type I gel in the µ-Slide Chemotaxis, migrating towards fetal calf serum. Note: the cells connecting to each other form strings during the chemotaxis process. Phase contrast, 4x objective lens, 24 hours.



Click here to watch the movie on our website.

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.



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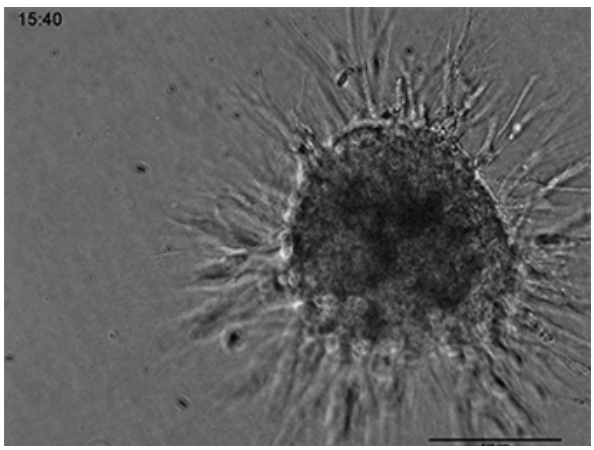
Sprouting of Endothelial Cells in a 3D Collagen Gel

Live cell imaging of a spheroid of Human Umbilical Vein Endothelial Cells (HUVEC), embedded into a 3D gel made of Collagen Type I, Rat Tail. The sprouting process into the gel matrix was recorded for 44 hours in the µ-Slide 8 Well. 10x and 4x objective lens, brightfield.



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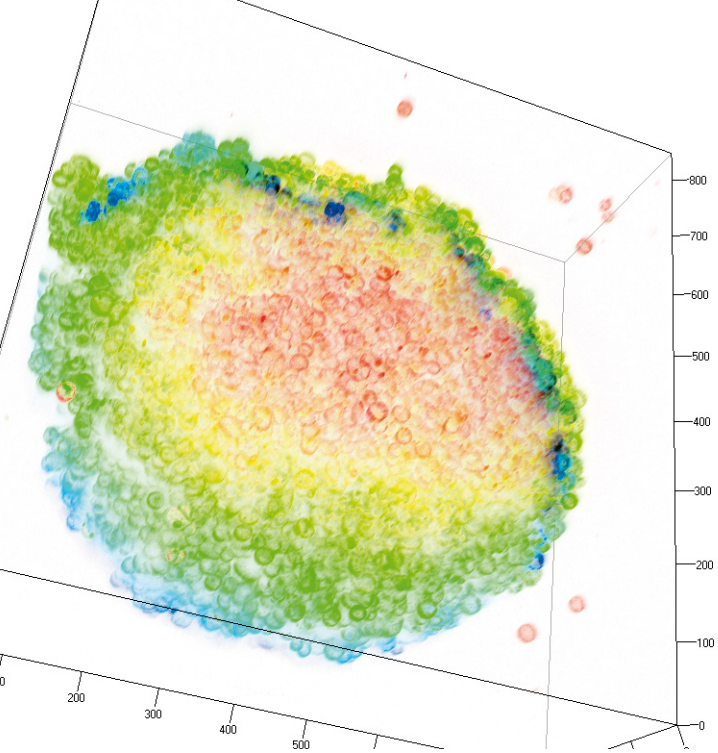

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