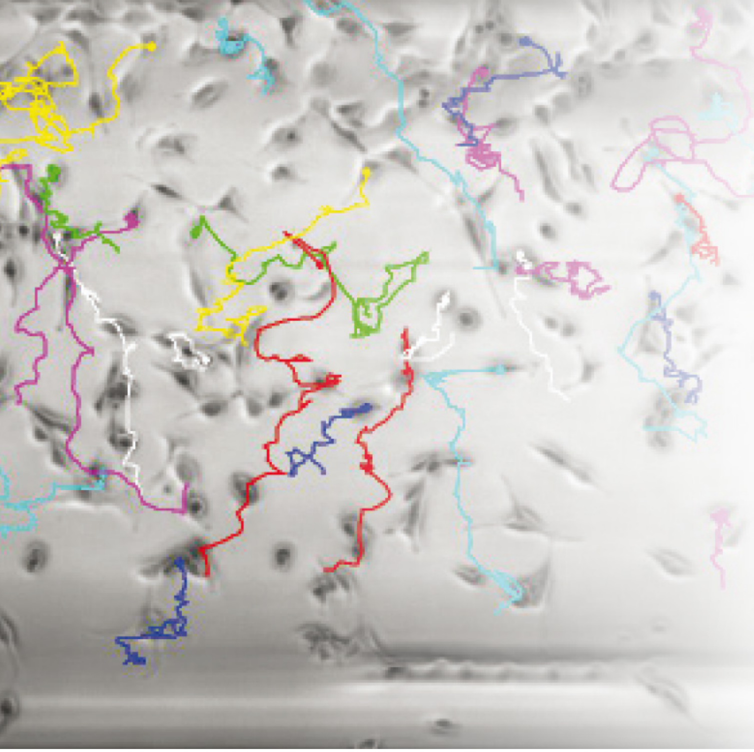

cells in focus ibidi®

ibidi Application Guide

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Selected Publications

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 abstract

L. Laganenka, R. Colin and V. Sourjik. Chemotaxis towards autoinducer 2 mediates autoaggregation in Escherichia coli. Nature communications, 2016, 10.1038/ncomms12984.
read abstract

P. Suraneni, B. Rubinstein, J. R. Unruh, M. Durnin, D. Hanein and R. Li. The Arp2/3 complex is required for lamellipodia extension and directional fibroblast cell migration. The Journal of Cell Biology, 2012, 10.1083/jcb.201112113.
read abstract

Y. Harada et al. DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses. Blood, 2012, 10.1182/blood-2012-01-407098.
read abstract

Chemotaxis

The Role of Chemotaxis in Cell Physiology

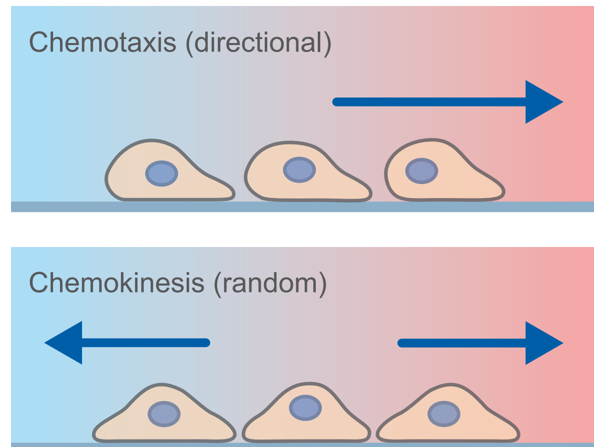
Chemotaxis is described as the directed migration of cells towards a chemoattractant. This process is different from chemokinesis, which is undirected cell migration. When cells undergo chemokinesis, they alter their migrational properties (e.g., increase or decrease of migration speed) due to a substance, but do not migrate preferably in the direction of that substance.

Chemotaxis is induced by a specific substance, the **chemoattractant**. This substance can be a chemokine, a chemokine receptor, a growth factor, or a growth factor receptor. Important and well-described chemoattractants are, for example, the chemokine receptor CXCR4 and its ligand CXCL12, EGFR and EGF, and also CCR7 and CCL21.

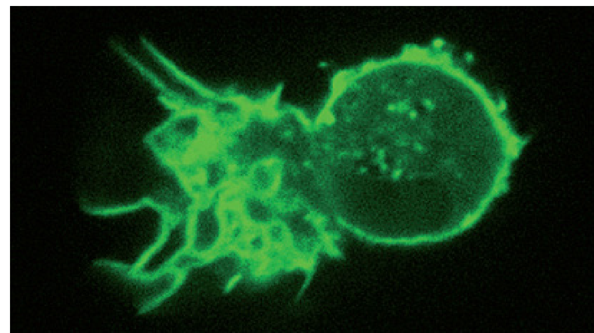
The chemotactic network plays an **important role in health and disease**. On the one hand, chemotaxis is crucial in many physiological processes, such as during the recruitment of inflammatory cells or organ development. On the other hand, chemotactic processes can promote cancer progression, such as during the metastatic process, when malicious reprogramming of chemotaxis leads to cell invasion and dissemination.

Tumor cells and their microenvironment constantly act in a complex communication network. Factors produced by epithelial tumor cells stimulate various immune cells, such as tumor-associated macrophages (TAMs), neutrophils, lymphocytes, and cancer-associated fibroblasts (CAFs). Upon stimulation, immune cells migrate towards the gradient, which can result in a tumor-suppressing or tumor-promoting immune answer.

Chemotaxis is also strongly involved in **angiogenesis**—an essential process that occurs during organ development and cancer progression. Angiogenesis helps to supply the tumor with oxygen and nutrients and is required for tumor dissemination. Tumor cells produce chemokines that influence the migration of endothelial cells, resulting in formation of the blood vessels. Further, chemokines can attract angiogenesis-promoting immune cells.



Chemotaxis-related migratory responses.



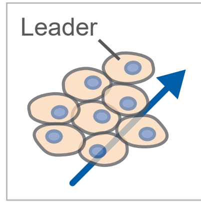
Murine dendritic cell with LifeAct-labeled F-actin that is migrating towards CCL19 inside a Collagen I gel matrix. Spinning disc confocal microscopy (objective lens 63x).

Trepat X, Chen Z, Jacobson K (2012) Cell migration. Compr Physiol 2(4):2369–92.
read abstract

Roussos ET, Condeelis JS, Patsialou A (2011) Chemotaxis in cancer. Nat Rev Cancer 11(8):573–587.
read abstract

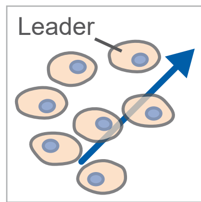
Types of Chemotaxis

Multicellular (e.g., Endothelial Cells)



Collective Migration

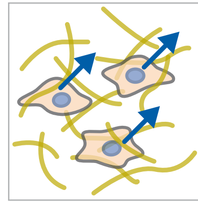
- Cell-cell junctions present, more than 2 cells in a group
- Leader cells create tracks, further cells follow
- Stromal cells involved



Cell Streaming

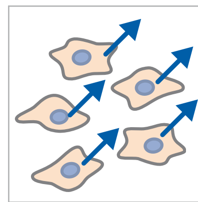
- Individual cells move and follow each other
- No cell-cell junctions required
- Stromal cells involved

Single Cells



Amoeboid Migration

- Fast cell migration (e.g., leukocytes 4 µm/min)
- Cells squeeze through extra-cellular matrix (ECM) gaps
- Much contractile force used, no matrix metalloproteinases (MMPs) required



Mesenchymal Migration

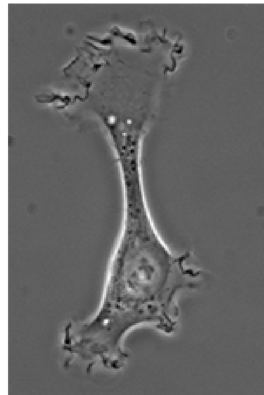
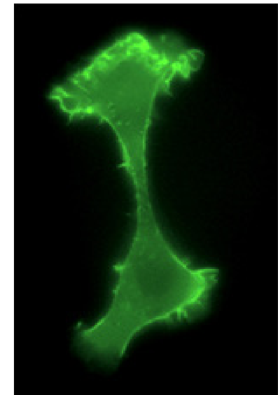
- Slow cell migration (e.g., fibroblasts 0.1–1 µm/min)
- Established cell polarity
- Elongated cells
- Depends on ECM proteolysis

Chemotaxis Assays

A chemotaxis assay is conducted to analyze whether or not a cell type directly orients and migrates towards a defined chemoattractant. Frequently used cell types in chemotaxis assays are cancer cells and immune cells.

Chemotaxis assays enable the investigation of:

- Chemotactic behavior of cells after knockout, knock-down, or overexpression of the gene of interest
- Chemotactic potential of substances
- Effect of chemotaxis inhibitors and enhancers
- Differentiation between chemotaxis and chemokinesis (enhanced migration)
- Cellular signal transduction during chemotaxis
- Changes in the cytoskeleton during chemotaxis

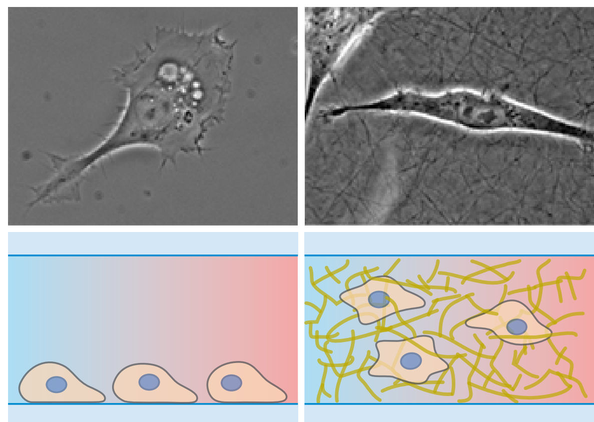



A chemotactic HT-1080 LifeAct-TagGFP2 cell migrating on a 2D surface.

2D Versus 3D Chemotaxis Assays

Many cells naturally grow in a three-dimensional environment. *In vitro*, cells are attached to a flat 2D surface and might behave differently than when they are inside a 3D gel matrix. This should be considered when planning chemotaxis assays.

Using the µ-Slide Chemotaxis, chemotactic gradients can easily be established in water-based gels, such as Collagen I gels and Matrigel, because the gel structure does not hinder the diffusion. Find out more information about the advantages and disadvantages of 3D chemotaxis assays on our website.



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

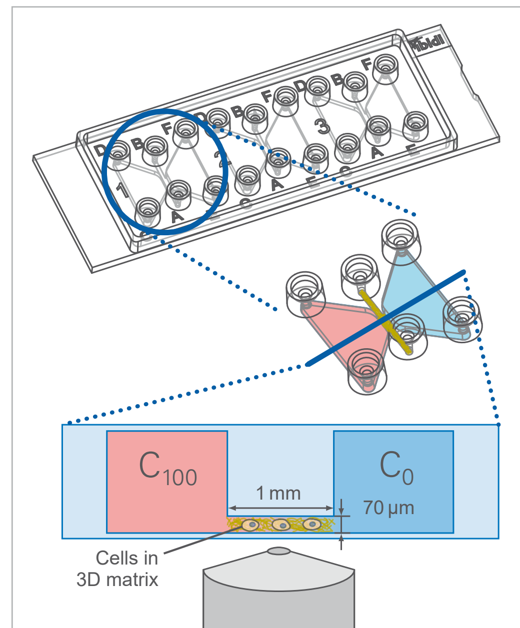
The ibidi µ-Slide Chemotaxis: The Ideal Chamber for Chemotaxis Assays

Principle and Applications

- In the ibidi µ-Slide Chemotaxis, cells migrate and can be observed in a central channel, which connects two large reservoirs
- Defined linear gradient with long-term stability
- Homogeneous cell distribution at the experimental starting point
- Ideal for live cell imaging using inverted microscopy
- Suitable for: - 2D and 3D assays
  - Fast and slow migrating cells
  - Adherent and non-adherent cells

The µ-Slide Chemotaxis was developed to investigate the chemotactical behavior of fast or slow migrating, non-adherent or adherent cells on 2D surfaces or in 3D gel matrices.

Using the µ-Slide Chemotaxis allows for detailed and defined analysis of the migration behavior of various cell types, such as endothelial cells, fibroblasts, cancer cells, and immune cells.

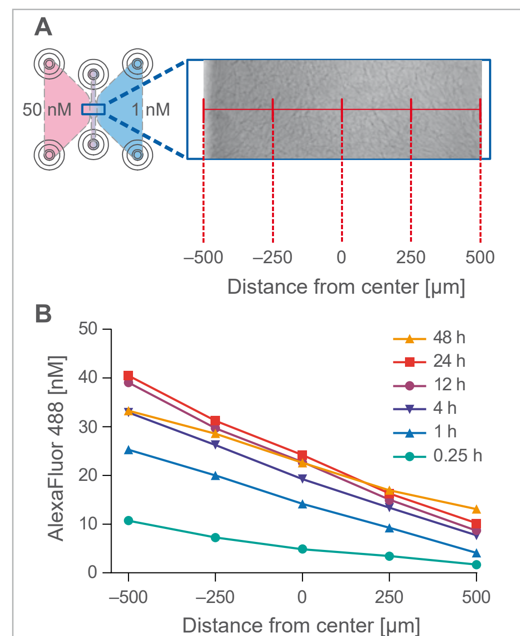


Using the µ-Slide Chemotaxis, the cells can be cultivated in a 3D gel matrix or on a 2D surface. In this example, a 3D experiment with migratory cells in a gel matrix is shown.

Gradient Stability

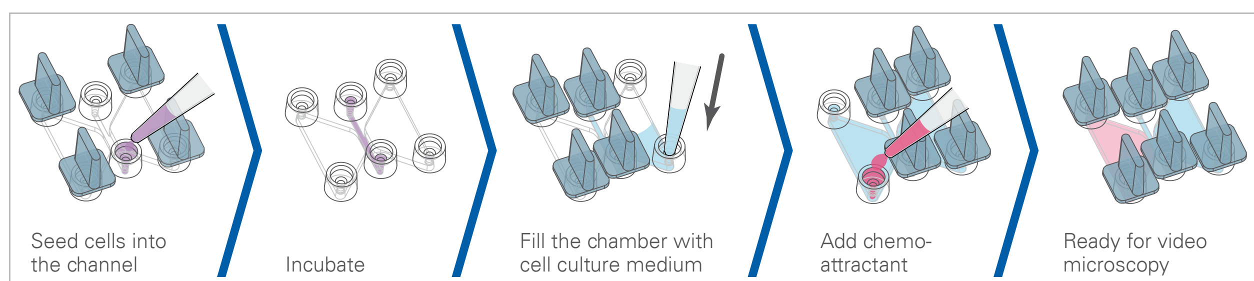
The ibidi µ-Slide Chemotaxis is designed to provide a quick gradient with excellent long-term stability for more than 48 hours. As gradients are rapidly established, fast migration responses (occurring in less than 30 minutes) can also be measured.

In 3D chemotaxis assays using aqueous gels, such as Collagen I or Matrigel, the gradient is stable and not influenced by the gel in any way.



Time-stable gradients across the observation area in the µ-Slide Chemotaxis. (A) Top view of the chemotaxis chamber with a collagen I matrix in the observation area. The reservoirs were filled with 50 nM and 1 nM AlexaFluor 488, respectively. Measurements were performed at five different x-positions, indicated in red. (B) Time-stable linear AlexaFluor 488 concentration profiles were created within the migration chamber, measured by fluorescence correlation spectroscopy.

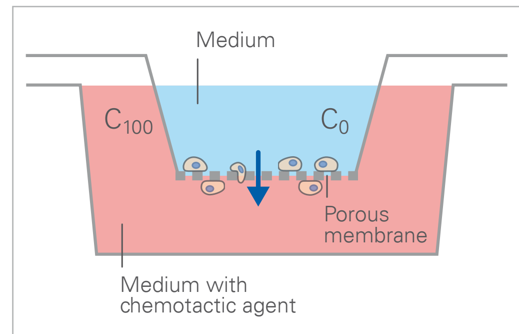
Sample Preparation



Further Chambers for Chemotaxis Assays

Boyden Chamber

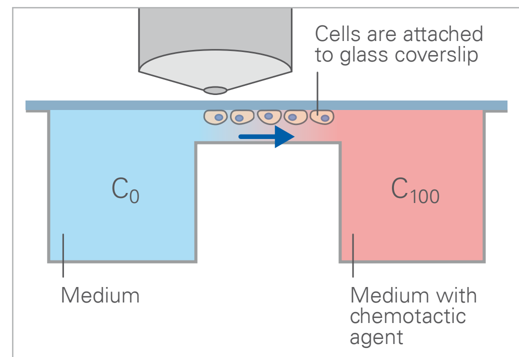
- Cells migrate from one side to the other side of a filter or a porous membrane
- Endpoint assay: migrating cells are counted at the back side of the membrane, and therefore cell paths cannot be analyzed
- Steep gradients
- Inhomogeneous cell distribution at the experimental starting point; cells are situated on one membrane side only



Boyden SV (1962) The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. J Exp Med 115(3):453–66. 10.1084/jem.115.3.453.
[read abstract]

Zigmond Chamber

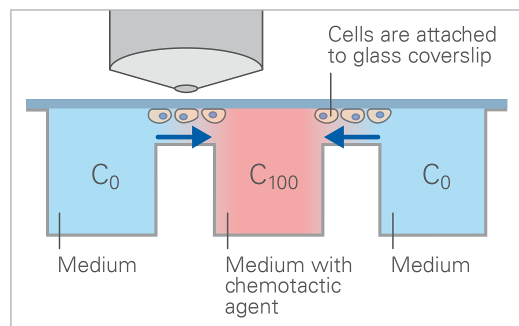
- Cells grow and migrate on a coverslip glass within a region of a bridge, which is located between two connected reservoirs
- Defined linear gradient
- No long-term stability
- Suitable for upright time-lapse microscopy
- Homogeneous cell distribution at the experimental starting point



Zigmond SH (1977) Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. J Cell Biol 75(2 Pt 1):606–16. 10.1083/jcb.75.2.606.
[read abstract]

Dunn Chamber

- Similar to the Zigmond chamber
- Defined linear gradient
- No long-term stability
- Suitable for upright time-lapse microscopy
- Homogeneous cell distribution at the experimental starting point

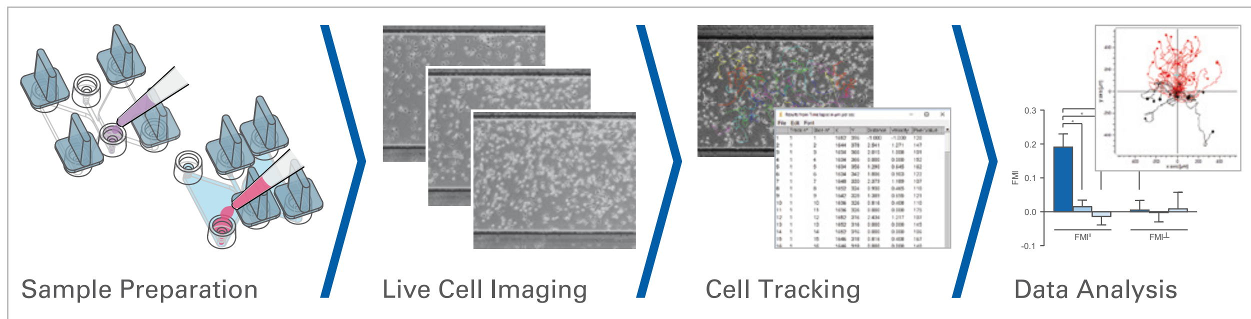


Zicha D, Dunn GA, Brown AF (1991) A new direct-viewing chemotaxis chamber. J Cell Sci 99(4).
[read abstract]

Zengel P, et al. (2011) µ-Slide Chemotaxis: a new chamber for long-term chemotaxis studies. BMC Cell Biol 12:21. 10.1186/1471-2121-12-21.
[read abstract]

Biswenger V, et al. (2018) Characterization of EGF-guided MDA-MB-231 cell chemotaxis in vitro using a physiological and highly sensitive assay system. PLoS One 13(9):e0203040. 10.1371/journal.pone.0203040.
[read abstract]

Experimental Workflow of a Chemotaxis Assay



Before Starting

Necessary Equipment

Basic requirements

- Inverted phase contrast microscope (5x or 10x objective recommended)
- Camera for time lapse image acquisition
- Stage Top Incubator (required for most mammalian cell types)

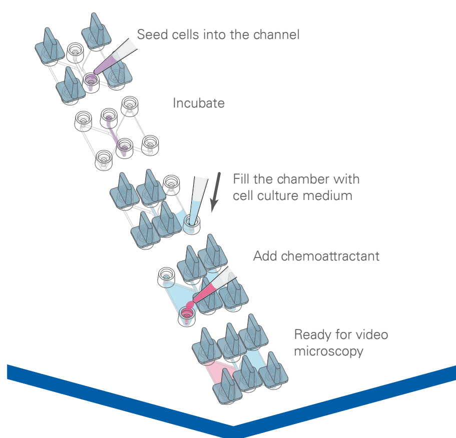
Recommended extensions

- Motorized stage for parallel image acquisition
- Autofocus

Questions to Ask

For a successful chemotaxis experiment, please address the following questions before starting.

Sample Preparation



Procedure

Using the µ-Slide Chemotaxis, reproducible chemotaxis assays with defined chemotactic gradients can be carried out.

First, the cells are seeded, which can be done either in a 2D or a 3D environment. After incubation and cell attachment, the two reservoirs of each chamber are filled with the chemoattractant, according to the determined loading scheme.

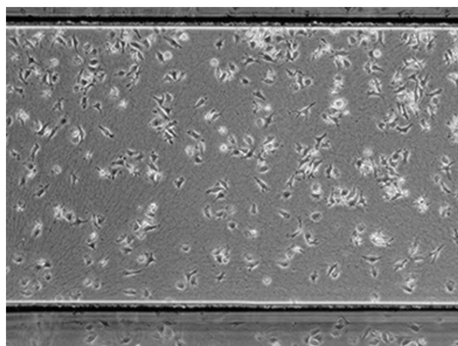
More information about the detailed handling of the µ-Slide Chemotaxis can be found in the Application Note AN 17: Chemotaxis 2D and 3D (PDF).

ibidi Solution

The µ-Slide Chemotaxis contains 3 separate chambers for parallel chemotaxis assays using slow or fast migrating cells.

It allows for the creation of a precisely defined, stable chemotactic gradient in a reproducible environment.

Live Cell Imaging



Time-lapse microscopy of the migration of MDA-MB-231 human breast adenocarcinoma cells in a collagen gel. Cells were observed over a period of 24 hours in the µ-Slide Chemotaxis with EGF as chemotactic agent. Physiologic conditions were maintained by the ibidi Heating and Gas Incubation System.

Procedure

Live cell imaging under physiologic conditions enables for the detailed documentation of the cell migration over time, a process which is necessary for the proper analysis of a chemotaxis assay.

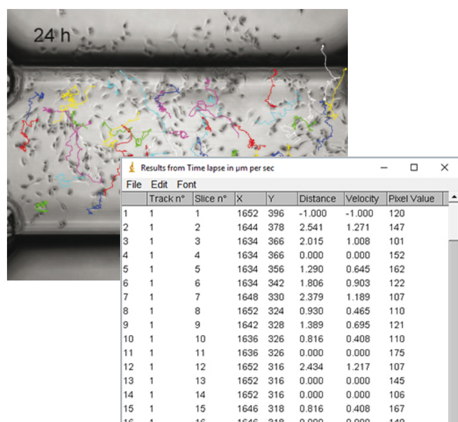
The duration of the imaging period depends on the cell type (e.g., fast migrating leukocytes or slow migrating tumor cells or fibroblasts) and the environmental conditions (e.g., type and concentration of the chemotactic agent). A typical video microscopy setup for slow migrating cells includes taking a photo every 2.5–10 minutes over a period of 24 hours.

Since each experiment optimally contains tracking data from 20–40 single cells, low-magnification microscopy objective lenses, such as 4x or 10x, should be used.

ibidi Solution

The ibidi Stage Top Incubation System provides a physiological environment under the microscope, which enables live cell imaging during short-term and long-term chemotaxis assays.

Cell Tracking



	Track n°	Slice n°	X	Y	Distance	Velocity	Pixel Value
1	1	1	1652	396	-1.000	-1.000	120
2	1	2	1644	378	2.541	1.271	147
3	1	3	1634	366	2.015	1.008	101
4	1	4	1634	366	0.000	0.000	152
5	1	5	1634	356	1.290	0.645	162
6	1	6	1634	342	1.806	0.903	122
7	1	7	1648	330	2.379	1.189	107
8	1	8	1652	324	0.930	0.465	110
9	1	9	1642	328	1.389	0.695	121
10	1	10	1636	326	0.816	0.408	110
11	1	11	1636	326	0.000	0.000	175
12	1	12	1652	316	2.434	1.217	107
13	1	13	1652	316	0.000	0.000	145
14	1	14	1652	316	0.000	0.000	106
15	1	15	1646	318	0.816	0.408	167
16	1	16	1646	318	0.000	0.000	140

Visualization of cell traces after tracking with the ImageJ Manual Tracking plugin.

Output of the ImageJ Manual Tracking Plugin after tracking; data table with positional values of each tracked cell (x, y) for each time point (t).

Procedure

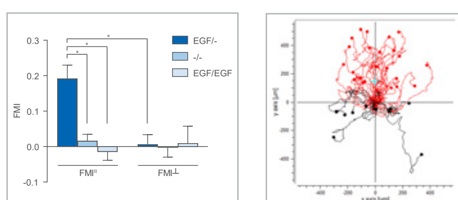
Typically, live cell microscopy of a chemotaxis assay creates a temporal image stack, where each image displays the exact cell position at a specific time point.

For quantification of their movement, single cells are tracked over time by determining their position on each frame of the image stack. The tracking can be done either manually or automatically using special software. Afterwards, the positional values of each tracked cell (x, y) are available for each time point (t) and can be further analyzed.

ibidi Solution

- Manual tracking: The ImageJ Manual Tracking Plugin assists in manually tracking cell migration in chemotaxis assays. It can be downloaded for free on our website.
- Automated tracking: MetaVi Labs—the ibidi partner for quantitative image analysis—is currently developing an automated solution to quantitatively evaluate 2D chemotaxis and migration. If you are interested to include your data into our beta testing program, please contact us at: acas@ibidi.com.

Data Analysis



Find a detailed description of the data analysis steps of chemotaxis assays here:

- Data Plotting
- Quantitative and Statistical Analysis
- Data Interpretation

Questions to Ask Before Starting an Experiment

In order to set up a chemotaxis assay correctly, it is crucial to answer the following questions first:

Which cell type are you planning to investigate?

Knowing your cell type of interest—including the culture medium requirements and the migration speed—is essential for subsequent assay planning.

What is the migration speed / velocity of the cell type being analyzed?

Whereas some cells have a low migration speed (e.g., tumor cells or fibroblasts), other cell types (e.g., leukocytes) migrate very quickly. The cell migration speed will determine the duration of the experiment and the intervals required between the images. Also, the gradient stability must be high enough to fit the total duration of the experiment. The µ-Slide Chemotaxis is suitable for chemotaxis assays with both slow and fast migrating cells.

	Slow migrating cells	Fast migrating cells
Migration type	Mesenchymal	Amoeboid
Examples	Endothelial cells, cancer cells, fibroblasts, stem cells	T cells, neutrophils, dendritic cells, Dictyostelium discoideum
Speed	One cell length per hour: ~10 µm/h	One cell length per minute: ~10 µm/min
Frame rate (time lapse)	1 image per 10 min	1 image per 30 s
Typical experiment duration	12–24 h	15–30 min

What is the optimal culture medium?

Most cell types are cultured in a medium supplied with fetal calf serum (FCS). FCS contains many factors (e.g., enzymes, hormones and growth factors) that can influence the cell migration, and therefore might alter the outcome of a chemotaxis assay. For example, the effects of a chemotactic agent on cell migration can be masked by FCS-induced effects. Gradual reduction of the FCS concentration in the medium, before starting the assay, is one way to overcome this issue. Furthermore, special, serum-free culture media without FCS have been developed.

The optimal medium composition for each cell type of interest must be tested before starting the assay.

What is the optimal seeding density for the cell type of interest?

The seeding density depends on many cellular factors, such as the proliferation rate, behavior, shape, epithelial or mesenchymal state, and the dependence on cell-cell contacts. In addition, the experiment duration must be considered when determining the optimal cell seeding density. In order to have enough trackable cells, the density must not be too low when starting the experiment. At the endpoint of the experiment, single cells should be clearly definable and trackable.

Considering these factors, the optimal seeding density should be separately determined for each cell type before starting the chemotaxis assay.

How many experiments should be performed?

Typically, three to five repeat experiments are sufficient to create significant data from the chemotaxis group and the respective control group. Each experiment should contain tracking data from 20–40 single cells, which is possible using low-magnification microscopy objective lenses, such as 5x or 10x.

Should I perform a 2D or a 3D chemotaxis assay?

Most cells are naturally embedded in a 3D matrix. Culturing them in a 2D environment during a chemotaxis assay might alter their behavior and migrational capabilities. To overcome this issue, cells can be embedded in a 3D matrix that mimics their natural environment, such as collagen, Matrigel, or other hydrogels. The µ-Slide Chemotaxis is ideally suitable for both 2D and 3D experiments.

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

Disadvantages 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:

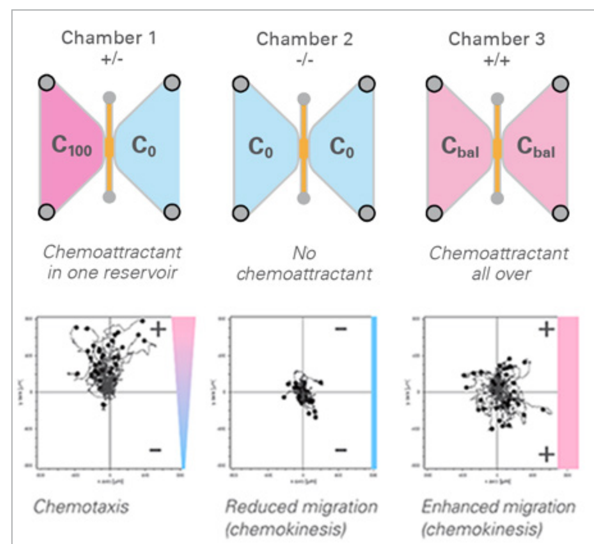
- AN 17: Chemotaxis 2D and 3D (PDF)
- AN 23: 3D Chemotaxis Protocol for Non-Adherent Cells in a Gel Matrix (PDF)
- AN 24: Chemotaxis of HT-1080 Cells in 2D and 3D (PDF)
- AN 26: Collagen I Gel for 3D Cell Culture (PDF)
- AN 34: Chemotaxis of HUVEC Cells in 2D and 3D (PDF)

Which controls must be included in the experiment?

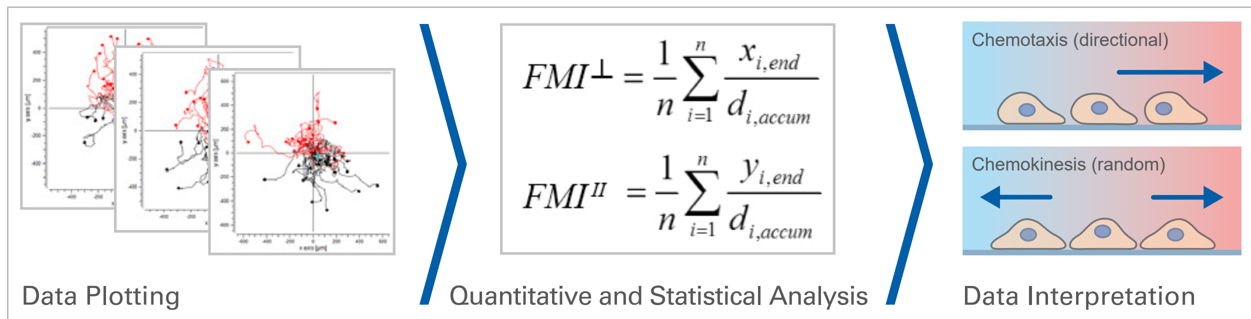
For the correct analysis of a chemotaxis experiment, it is crucial to include a negative control without any chemoattractant (-/-), as well as a positive control with chemoattractant over the entire chamber (+/+).

Using the µ-Slide Chemotaxis, a minimum of control measurements are required, since all conditions other than the gradient are symmetric. This allows for the analysis of chemotaxis and chemokinesis independently of each other.

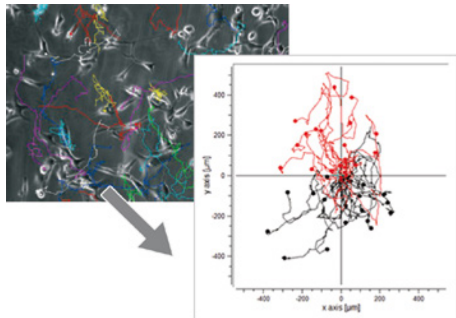
In the example, the chemoattractant induces both the chemotaxis and chemokinesis of cancer cells.



Data Analysis of Chemotaxis Assays



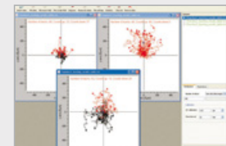
Data Plotting



After tracking, the paths of the cells can be visualized in trajectory plots that will already give information about the chemotactic effects. This is usually done using special software.

In a trajectory plot, all (x,y) coordinates of the cells' starting points are set to (0,0) using a coordinate transformation. With this setting, the general direction of cell migration is displayed and allows for first data interpretations. To finally confirm whether or not the observed cell migration is chemotactic or random, the data must be statistically analyzed.

ibidi Solution



The Chemotaxis and Migration Tool allows for the fast and easy creation of cell trajectory plots, as well as subsequent statistical analysis.

For a better visualization of the chemotaxis effects, additional information can be displayed in the plots (e.g., color information of cells moving up/down, center of mass, and cell endpoints). All data can be exported for further analysis. The tool is freely available for download on our website.

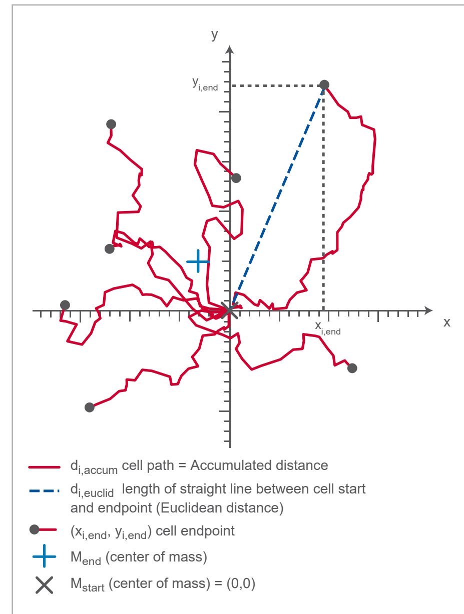
Quantitative and Statistical Analysis

In order to analyze and interpret chemotaxis assays correctly, it is necessary to know the meaning and calculation of the relevant chemotaxis parameters.

The most important parameter for quantifying chemotactic cell migration is the Forward Migration Index ($FMI^{\perp}$, FMI^{II}). A further measure of directed cell migration is the Center of Mass (M_{end}). Additionally, we recommend the Rayleigh test, which statistically determines if the cell distribution is homogeneous or inhomogeneous.

Further migration parameters are the Directness (D) and the cell speed/velocity, which, however, may not necessarily indicate a chemotaxis effect. All these relevant chemotaxis values can be automatically calculated by using the Chemotaxis and Migration Tool.

Zengel P, et al. (2011) µ-Slide Chemotaxis: a new chamber for long-term chemotaxis studies. BMC Cell Biol 10.1186/1471-2121-12-21.
read abstract



Chemotaxis plot displaying important chemotaxis parameters.

Forward Migration Index ($FMI^{\perp}$, FMI^{II})

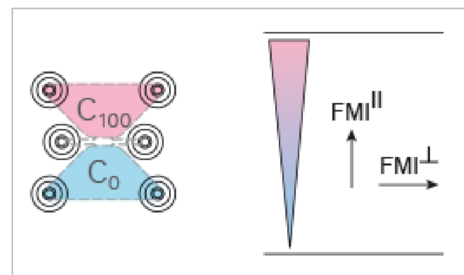
The Forward Migration Index (FMI) is an important measure for directed, chemotactic cell migration. It represents the efficiency of the forward migration of cells.

For FMI calculation, it is necessary to define the direction of the x-axis and the y-axis in relation to the chemotactic gradient. Here, we define the x-axis as perpendicular to the gradient (⊥) and the y-axis as parallel to the gradient (II).

To determine if directed cell migration occurs in a chemotaxis assay, two FMI values have to be calculated:

- $FMI^{\perp}$ (x_{FMI}) describes the forward migration of cells in a direction perpendicular to the gradient
- FMI^{II} (y_{FMI}) describes the forward migration of cells in a direction parallel to the gradient

The larger the $FMI^{\perp}$ or the FMI^{II}, the stronger the chemotactic effect is on the x-axis or y-axis, respectively.



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

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

Calculation of the $FMI^{\perp}$ and the FMI^{II}. i = index of single cells, n = number of cells, $x_{i,end}$, $y_{i,end}$ = coordinates of the cells' endpoints, $d_{i,accum}$ = accumulated distance of the cells' paths.

Rayleigh Test

The Rayleigh test is a statistical test for the uniformity of a circular distribution of points (cell endpoints).

With $p < 0.05$, the null hypothesis (uniformity) is rejected, indicating a chemotaxis effect. Like all statistical tests, this one strongly depends on the number of analyzed cells. This Rayleigh test for vector data also includes the distance from the origin.

Moore BR., 1980, A modification of the Rayleigh test for vector data, Biometrika, Volume 67, 175-180

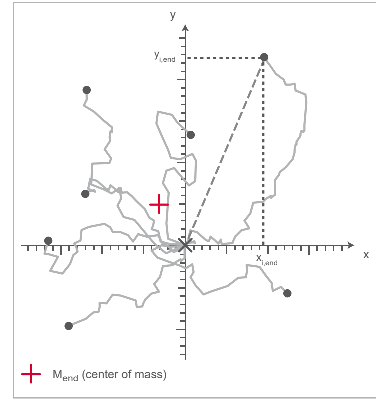
Center of Mass (M_{end})

The center of mass represents the average of all single cell endpoints. Its x and y values indicate the direction, in which the group of cells primarily traveled.

Moore BR., 1980, A modification of the Rayleigh test for vector data, Biometrika, Volume 67, 175-180

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

Calculation of the center of mass (M_{end}). i = index of single cells, n = number of cells, $x_{i,end}$, $y_{i,end}$ = coordinates of the respective cell endpoint.



Chemotaxis plot; the Center of Mass is indicated in red.

Directness (D)

The directness (sometimes also called directionality) is a measure of the straightness of the cell trajectories.

It is calculated by comparing the Euclidean distance (di,euclid) and the accumulated distance (di,accum) between the starting point and the endpoint of a migrating cell.

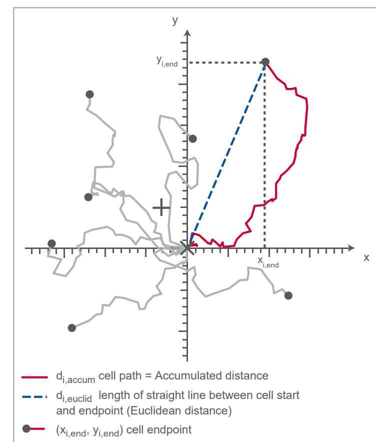
The directness values are always positive. A directness of D = 1 equals a straight-line migration from the start to the endpoint.

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

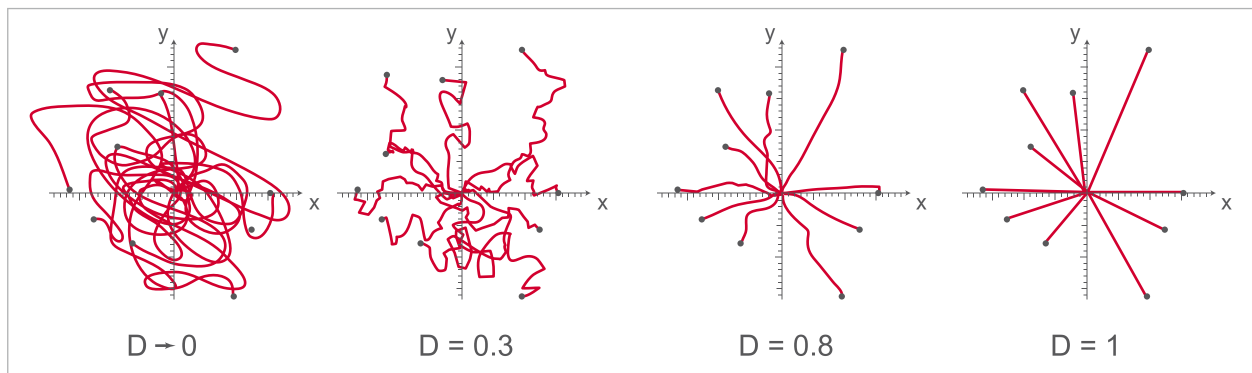
Calculation of the directness (D) of one single cell. i = index of the single cell, $d_{i,euclid}$ = Euclidean distance, $d_{i,accum}$ = accumulated distance.

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

Averaged directness of all cells in an experiment.



Chemotaxis plot indicating $d_{i,accum}$ (red) and $d_{i,euclid}$ (blue), which are important to calculate the directness (D).



Four examples of cell directness. A directness value approaching 0 indicates indirect, curvy cell migration. A directness value tending towards 1 indicates a straight migration from the start to the endpoint. Note, that the cell endpoints are identical in this example.

ibidi Solution

The Chemotaxis and Migration Tool provides chemotaxis plots, graphs, and statistical tests for the advanced analysis of chemotaxis assays. All relevant chemotaxis parameters are automatically calculated. All data can be exported for further analysis. The tool is freely available for download on our website.

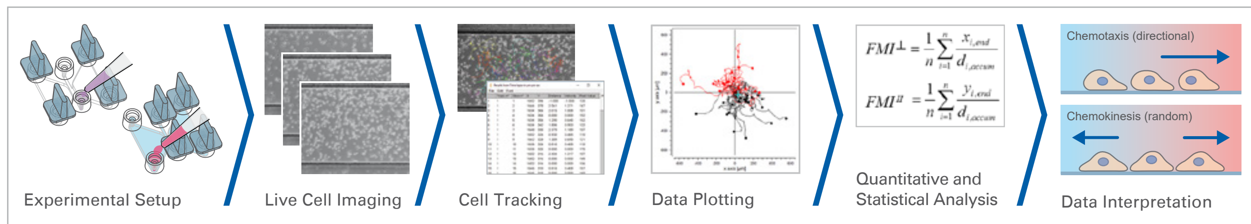
Data Interpretation

The following criteria must be fulfilled to determine directed, chemotactic cell migration and to exclude biased migration by non-chemotactic environmental factors:

- The $FMI^{\parallel}$ of the experimental group (+/-) must be significantly higher than the $FMI^{\parallel}$ of the respective positive (+/+) and negative (-/-) control groups
- The $FMI^{\parallel}$ of the experimental group must be significantly higher than the corresponding $FMI^{\perp}$, which must be close to zero
- The $FMI^{\perp}$ and the $FMI^{\parallel}$ of the control groups (+/+, -/-) must be close to zero
- The Rayleigh test of the experimental group must result in $p < 0.05$ (significant)
- The Rayleigh test of the control groups must result in $p > 0.05$ (not significant)

If these criteria are not fulfilled, the cell migration must be considered as random and non-directed.

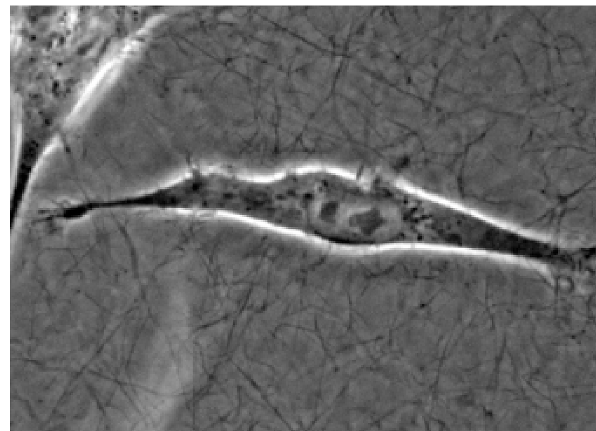
Example Data of a Chemotaxis Assay



We here provide all files that were created during a chemotaxis experiment for download, to give you the ability to practice every step of the analysis and interpretation of chemotaxis data.

In this example 3D chemotaxis assay with the MDA-MB-231 human breast adenocarcinoma cell line using the μ-Slide Chemotaxis, the epidermal growth factor (EGF) was used as chemotactic attractant.

Besides the experimental group (EGF/-), a negative (-/-) and a positive (EGF/EGF) control group were included. The assay was performed using three biological repeats of each experimental group.

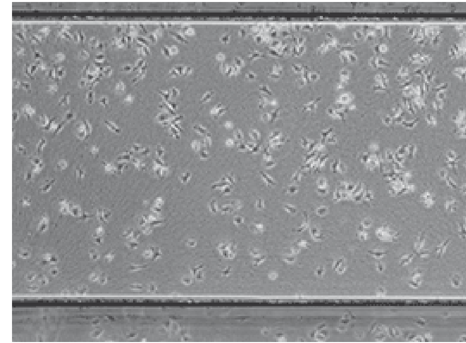


Microscopy of adherent HT-1080 cancer cells embedded into a 3D Collagen I gel in the μ-Slide Chemotaxis.

Experimental Setup

Cells	MDA-MB-231 (human breast adenocarcinoma cells)
Slide	μ-Slide Chemotaxis, ibiTreat (80326)
3D gel matrix	Collagen Type I, 1.5 μg/ml
Experimental medium	UltraCULTURE™ Serum-Free Medium (Lonza)
Attractant medium [EGF]	10 ng/ml EGF in UltraCULTURE™ Serum-Free Medium (Lonza)
Chamber 1	Experimental group (EGF/-)
Chamber 2	Negative control group (-/-)
Chamber 3	Positive control group (EGF/EGF)
Imaging mode	Phase contrast
Imaging period	24 h
Time interval	10 min
X/Y calibration (pixel size)	0.805 μm/px
Number of slices/images	145
Sample size	40 counted cell tracks per chamber, three biological repeats (one example for download)

Live Cell Imaging

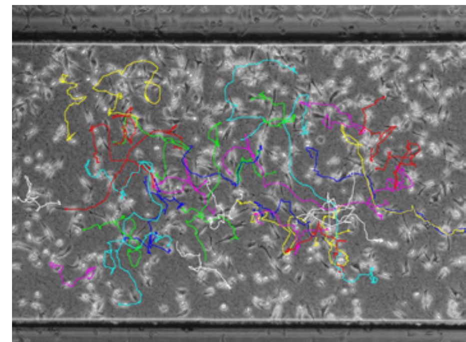


After live cell imaging, the following image stacks were obtained. Each stack contains 145 pictures, which were taken within a time period of 24 hours in 10 minute intervals. These images serve as raw data for subsequent cell tracking.

Download the image stacks of the example experiment from our website:

- EGF/- (experimental group)
- -/- (negative control group)
- EGF/EGF (positive control group)

Cell Tracking



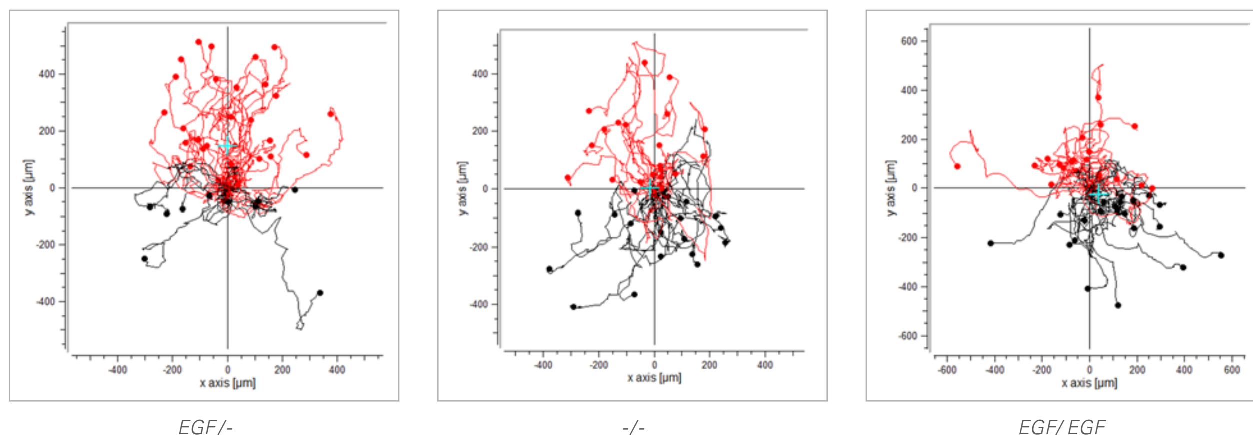
The following values were obtained by manual tracking using the ImageJ Manual Tracking Plugin (tab-delimited text). The files can be directly imported into the Chemotaxis and Migration Tool for further analysis.

Download the manual tracking results of the example experiment from our website:

- EGF/- (experimental group)
- -/- (negative control group)
- EGF/EGF (positive control group)

Data Plotting

Data plotting using the Chemotaxis and Migration Tool allows for a first visual inspection of the cell trajectories, including the cell velocity and directionality:



EGF/- *-/-* *EGF/ EGF*

Visualization of the cell migration in a chemotaxis assay, showing the experimental group (EGF/-), the negative (-/-), and the positive (EGF/ EGF) control group. Red cell trajectories show the migration towards the chemotactic agent, black cell trajectories indicate the migration in the opposite direction.

The EGF/- group shows cell migration in direction to the gradient. In contrast, the -/- and the EGF/EGF group show cell migration without any clear direction. In this experiment, the trajectory plots already indicate a chemotactic effect of the EGF. However, in order to confirm a chemotactic, direct cell migration towards the EGF and to exclude any environmental factors, a thorough statistical analysis of the chemotaxis parameters is mandatory.

Quantitative and Statistical Analysis

Statistical analysis of the example experiment using the Chemotaxis and Migration Tool generated the following values, which include all important chemotaxis parameters:

	EGF/– (experimental group)	–/– (negative control group)	EGF/EGF (positive control group)
Forward migration index, perpendicular, FMI⊥ [x_{FMI}]	0.014	-0.014	0.056
Forward migration index, parallel, FMI ll [y_{FMI}]	0.18	-0.00021	-0.025
Directness [D]	0.32	0.32	0.33
Center of mass x [x_{end}, µm]	1.39	-15.78	38.56
Center of mass y [y_{end}, µm]	146.19	1.83	-25.38
Center of mass length [M_{end} µm]	146.2	15.88	46.16
Rayleigh test (p-value)	0.0012	0.79	0.34
Mean accumulated distance [d_{accum}, µm]	799.89	665.8	730.94
Mean Euclidean distance [d_{euclid}, µm]	260.08	211.27	235.39
Cell velocity [µm/min]	0.56	0.46	0.51

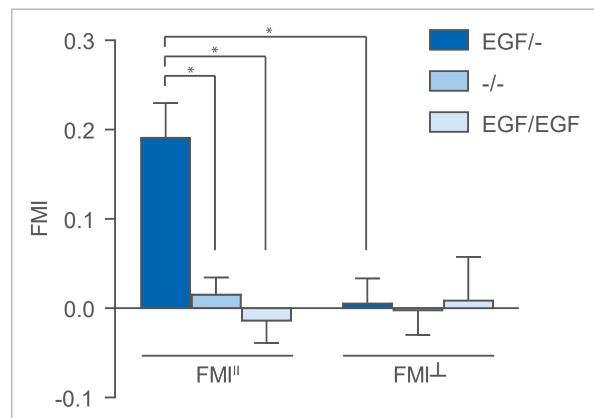
As described in the Quantitative and Statistical Analysis section of the Data analysis chapter, the relevant chemotaxis parameters are used to prove the chemotactic effect of an agent.

Download the measured values of the example experiment on our website.

Data Interpretation

In this experiment, the FMI ll of the EGF/- group is significantly higher than the FMI ll of the -/- and EGF/EGF control groups. Further, the FMI ll of the EGF/- group is significantly higher than the corresponding FMI⊥, which is close to zero. The FMI⊥ and the FMI ll of both control groups are close to zero as well. The Rayleigh test results in $p < 0.05$ in the EGF/- group and $p > 0.05$ in the control groups.

Taking all these criteria into account, the data analysis shows a significant chemotactic migration of the MDA-MB-231 cells in direction of the EGF.



Averaged FMI ll and FMI⊥ of the EGF/- experimental group in comparison to the -/- and the EGF/EGF control groups. Significant chemotaxis is observable parallel to the gradient in the EGF/+ group. Please note: this graph includes three biological replicates.

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