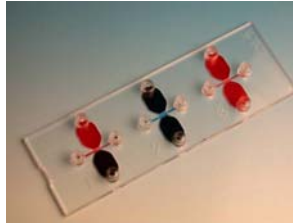


Product information

μ -Slides Chemotaxis



μ -Slide Chemotaxis



μ -Slide Chemotaxis^{3D}

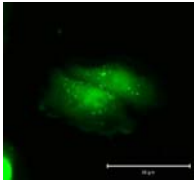
Chemotaxis is the directed movement of a cell, or organism, along a concentration gradient of a chemotactic agent. Chemotaxis is called positive if movement is in the direction of a higher concentration of the chemical in question, or negative if the direction is opposite.

Slow cells move with a speed of approx. 20 $\mu\text{m/hr}$ towards chemoattractant sources. Therefore movies need to be taken at least for 12 hours. The μ -Slide Chemotaxis is especially designed for this application.

Definition:

Directed movement of a cell or organism along a concentration gradient of a chemotactic agent

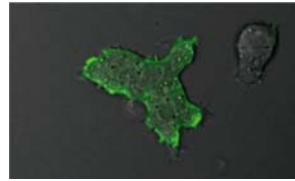
Slow migrating cells,
e.g. fibroblasts



~ 1 cell length / hour
(~20 $\mu\text{m}/\text{h}$)

Factor 10 – 100 !!!

Fast migrating cells,
e.g. leukocytes



~ 1 cell length / minute
(~20 $\mu\text{m}/\text{min}$)

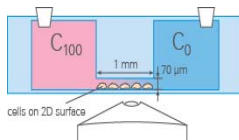
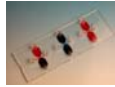
→ Time stable gradient needed (12 h)

→ Short time gradient needed (30 min)

**Chemotaxis slides are especially designed
for both applications.**

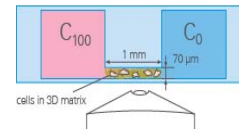
μ -Slide Chemotaxis

- Adherent cells
 - on 2D surface
 - in linear gradients
 - slow migrating cells
- Chemotaxis of cancer, endothelial cells, fibroblasts



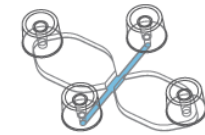
μ -Slide Chemotaxis^{3D}

- Non-adherent cells
 - in gel matrices
 - in linear gradients
 - fast or slow migrating cells
- Chemotaxis of neutrophils, lymphocytes, macrophages
- Interstitial chemotaxis of tumor and endothelial cells

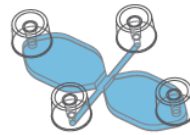




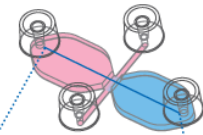
Preparation



seed cells in the cross channel



fill both reservoirs with cell free medium

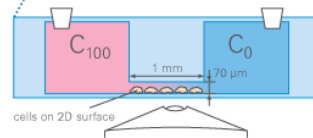


fill one of the reservoirs with chemoattractant

Basic principle

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

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



The basic concept of the μ -Slide Chemotaxis

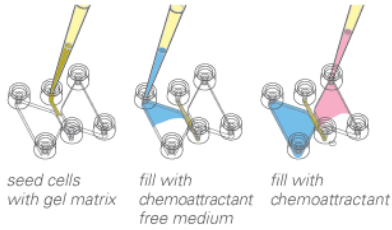
μ -Slide Chemotaxis consists of two reservoirs connected by a very thin observation volume - one reservoir filled with neutral solution, the other reservoir filled with the chemoattractant solution. The adherent cells are grown on the bottom of the observation area. Across the observation area (1 mm) a linear gradient of the chemoattractant solution is formed by diffusion. Specially designed plugs serve to hinder evaporation, and stabilize the diffusion gradient.

The dimensions of the μ -Slide Chemotaxis were calculated in a way that...

- Gradients will be stable for more than 48 hours
- Gradient steepness will be adequate for chemotaxis studies
- Volume of medium is sufficient for cell culture



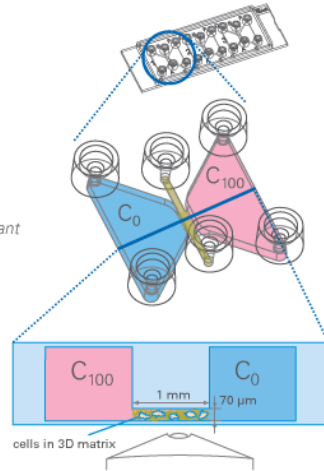
Preparation



Basic principle

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

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



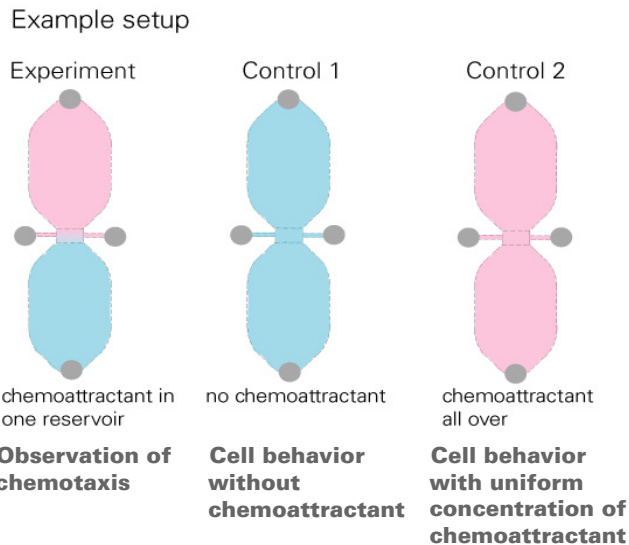
- Standard dimension of glass slide (25.5 x 75.5 mm²)
- High resolution fluorescence and confocal microscopy possible*
- For both slow and fast moving adherent cells
- Detailed movies on setup and application notes available

* For standard assay: Low magnification video microscopy with inverse microscope

The μ-Slide Chemotaxis was especially developed for video microscopy assays. At specific time intervals, pictures are taken from the observation area. The time interval between photo frames is dependant upon the speed of cell migration, such that the faster cells move, the more frames per minute are taken.

The majority of assays will be performed at low magnification (5x, 10x or 20x) because most parameters like speed or directionality are derived from the cells' positions only. However, high resolution microscopy is also possible. Therefore, also the co-localization or recruitment of proteins can be analyzed by fluorescence techniques. Although, we recommend inverted microscopes, upright microscopes may also be used with objectives at working distances larger than 12 mm.

- **Successful measurement in real-time:**
Stable gradients for long-term experiments
- **Fast:**
Ready-to-use system, 3 chambers on 1 slide
- **Easy analysis:**
Ideally suited for fluorescence microscopy
- **Reproducible results:**
Reliable and user-independent data



How to do the experiment

- Cells are seeded in the observation area
- The reservoirs are filled with the required solutions
- For building the gradient one reservoir must contain neutral medium – the other reservoir must contain chemoattractant
- The cells are imaged over an appropriate period of time (i.e. 12hr in case of many mammalian cells)

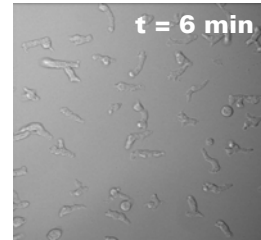
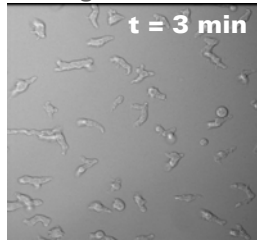
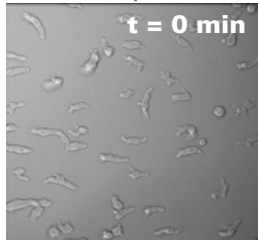
Reference (control) measurements

In addition to the measurement itself, two reference measurements must be performed. One reference measurement involves the use of chemoattractant in both reservoirs, the other uses neutral solution in both reservoirs. As the assay is of high significance, typically one to two reference measurements are sufficient.

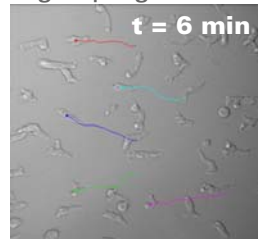
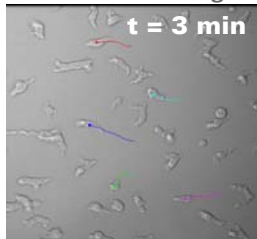
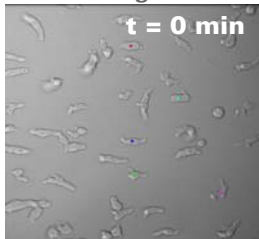
No chemoattractant: The normal cell behavior serves as reference in order to compare.

Uniform chemoattractant distribution: Important control measurement testing the general influence of the chemoattractant. For example, non directed cell motility is usually influenced by most chemoattractants.

1. Time lapse movie of moving cells

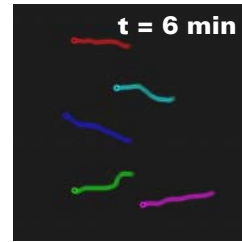
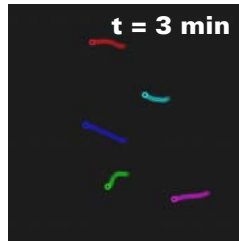
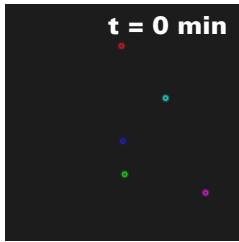


2. Tracking of cells: e.g. Manual Tracking with ImageJ plug in



The chemotaxis assay is based on video microscopy. Images are taken and put into a temporal stack. The cells from this stack are then tracked by appropriate software. We recommend Manual Tracking, a free ImageJ plugin or any other software tool capable of quantifying movements of objects between frames of a temporal stack.

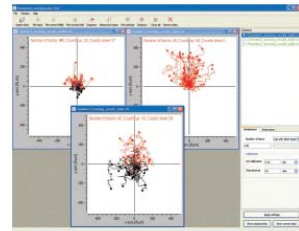
3. Plotting and Analyzing



Track n°	Start x	Start y
1	1	293 295
2	2	237 291
3	3	215 279
4	4	209 275
5	5	194 266
6	6	166 261
7	7	154 261
8	8	147 261
9	9	136 258
10	10	124 247
11	11	113 241
12	1	257 367
13	2	247 366
14	3	233 366
15	4	224 377

Data table

ibidi software:
 + "Chemotaxis and Migration Tool" =



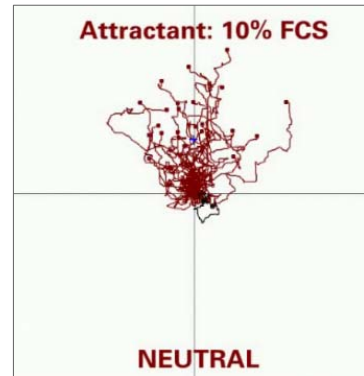
After tracking, the cells' paths can be exported and analyzed.

For data analysis ibidi provides a free ImageJ plugin named 'Chemotaxis and Migration Tool'. This tool can be downloaded from www.ibidi.com. It provides numerous features for proper migration analysis. Data files can easily be imported and converted into chemotactical plots. ibidi's plugin converts the cells' paths in a standard manner. All starting points of the cells are combined into the origin of a coordinate system. This representation of data can be analyzed "by eye". Migration characteristics also can be expressed in numbers.

Example: Mammalian cell motion



Chemotaxis of HT1080 cancer cells



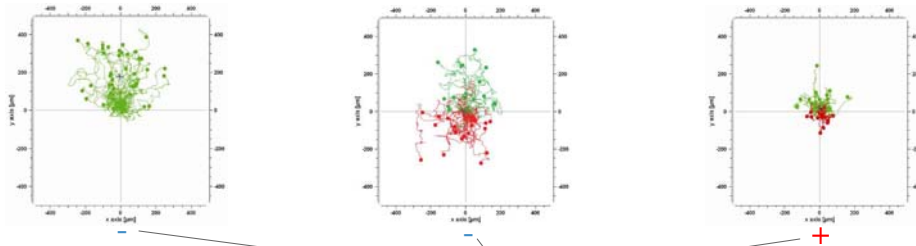
Watch video at:

http://www.ibidi.de/movies/MV_008_CT_HT1080.html

This example demonstrates the ability to observe slow migrating cells. The slower the cells move the longer the gradient needs to be maintained stable. With the unique time stability of over 48 hours, assays with μ -Slide Chemotaxis gain access to slow cells that have never been investigated so long before.

During 12 hours the 'center of mass' of all cells moves 63 μ m towards the source of chemoattractant.

Quantification of Results



Center of mass x [μm]	-4.0	3.6	-7.6
Center of mass y [μm]	176.3	18.1	-3.1
Center of mass length [μm]	176.4	18.5	8.2
X_{PM}	-0.014	0.015	-0.004
Y_{PM}	0.280	0.035	-0.007
Directionality D	0.33	0.16	0.21
Mean euclidian distance [μm]	208.9	65.8	145.8
Mean accumulated distance [μm]	617.1	411.8	716.4
Cell velocity [$\mu\text{m}/\text{min}$]	0.43	0.29	0.49
Rayleigh test	< 0.05	>0.05	>0.05

Chemotaxis Values

Here examples are given for parameters potentially used to characterize chemotaxis. All data can be automatically derived from ibidi's 'Chemotaxis and Migration Tool'.

- **Movies**

1. Preparation of μ -Slide Chemotaxis
2. Chemotaxis of HT1080 in μ -Slide Chemotaxis
3. Chemotaxis of HUVEC blocked by Spongistatin



- **Application Notes**

- 14: Chemotactic assay using μ -Slide Chemotaxis
- 17: Chemotaxis 3D
- 23: Chemotaxis of Dendritic cells

- **Scientific Publications**

- **ibidi Analysis Software**

Chemotaxis and Migration tool



Basic requirements

- (Inverted) microscope, objective 5 x, 10x phase contrast
- Basic camera for time lapse movies
- Heated microscopy stage (for most mammalian cells)

Recommended extensions

- Motorized stage (for parallel data acquisition)
- Auto focus
- CO₂ incubation system

The μ -Slide Chemotaxis in combination with video microscopy is able to collect high content data. For the standard assay low resolution microscopy is sufficient. A simple (digital) video camera basic acquisition software is sufficient. For most mammalian cell types a heated stage is required to keep the temperature stable at 37 °C.

To increase the data output we recommend the use of motorized stages and synchronized image acquisition. Together with low magnification motorized z-drive or auto focus is not necessary but advantageous. CO₂ incubation might be favorable for most cells.

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