Chemotaxis of HT-1080 cells in 2D and 3D

1. General Information

This is a detailed protocol for analyzing the chemotaxis of HT-1080 human fibrosarcoma cells (ATCC: CCL-121; DSMZ: ACC 315) using the µ-Slide Chemotaxis^{3D}. This slide can be used for two-dimensional (2D) as well as for three-dimensional (3D) experiments. Example data for cells cultured on a 2D surface, in bovine and rat tail collagen type I gels and exposed to an FCS gradient are given in this Application Note. More detailed handling information is provided in Application Note 17 "*3D Chemotaxis Assays using µ-Slide Chemotaxis ^{3D}*".

2. Equipment and Material Needed

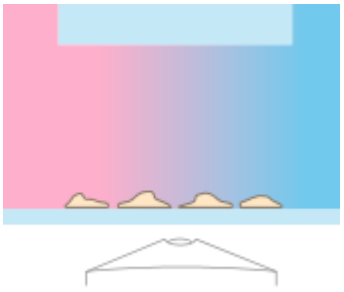
A live cell imaging set-up is a prerequisite for performing migration studies using the µ-Slide Chemotaxis^{3D}. A motorized stage is recommended to be able to observe all 3 chambers of one slide or even several slides at the same time. Detailed information about the hardware and the software needed can be found in Application Note 17.
The 2D as well as the 3D experiments were performed using the µ-Slide Chemotaxis^{3D} (ibidi, 80326). The 3D environment was provided by embedding HT-1080 cells in either bovine or rat tail collagen type I gels. A list of all needed ingredients for the gels and detailed protocols can be found in Application Note 26 "*Collagen I gel for 3D Cell Culture*".

3. Procedure

The µ-Slide Chemotaxis^{3D} was used for both 2D and 3D experiments. Detailed illustrated information about handling this slide, experimental planning and troubleshooting can be found in Application Note 17.

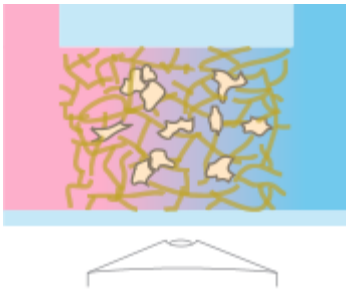
3.1. Performing 2D Chemotaxis Experiments

- Cell type: HT-1080
- Cell culture medium: DMEM + 10% FCS
- Cell culture surface: ibiTreat
- Final cell concentration: 3 x 10^6 cells/ml
- Attractant: 10% FCS
- Time lapse measurement: 24 h every 10 min
- Objective: 4x (phase contrast)



Prepare the cell suspension as usual and dilute it to the desired cell concentration. Pipette the cell suspension into the channel and let your cells attach to the surface. Start the time lapse measurement after filling medium and attractant in the reservoirs.

3.2. Performing 3D Chemotaxis Experiments



- Cell type: HT-1080
- Cell culture medium: DMEM + 10% FCS
- Cell culture surface: ibiTreat
- Final cell concentration: 3 x 10^6 cells/ml
- Attractant: 10% FCS
- Collagen Type I Gels: bovine and rat tail collagen gel
- Time lapse measurement: 24 h every 10 min
- Objective: 4x (phase contrast)

Prepare the cell suspension as usual and dilute it to the desired cell concentration. Mix the cell suspension and the collagen gel components thoroughly and apply it into the channel. After gelation and filling of the reservoirs, place your slide on the microscope and start the time lapse measurement.

Table 1 Composition of a 1.5 mg/ml bovine and rat tail collagen I gel. All ingredients are listed in order of pipetting. See Application Note 26 for the full protocol.

Ingredient	bovine (RT)	rat tail (one ice)
10x DMEM	20 µl	20 µl
NaOH 1M	6 µl	5 µl
H_2O	14 µl	81 µl
$NaHCO_3$ 7.5%	10 µl	4 µl
1x DMEM	50 µl	50 µl
Collagen I	150 µl	90 µl
Cell suspension	50 µl	50 µl
Total	300 µl	300µl

3.3. Chemotaxis Experiment Setup

A reliable experimental setting includes not only the chemotaxis experiment (+/-) itself, but also two control experiments (+/+, -/-). The latter are performed by filling the chamber completely with chemoattractant solution (+) or with chemoattractant-free (-) medium. These control experiments help to determine whether a compound is influencing the directed movement of cells and/or random migration.

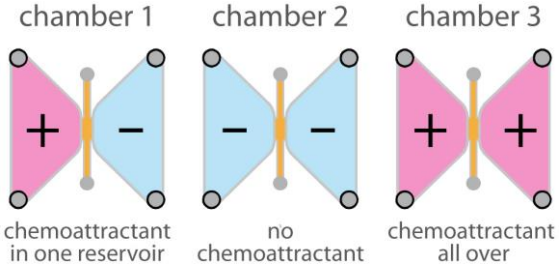


Figure 1 Recommended setup for one slide.

4. Examples

4.1. Comparison of 2D and 3D Chemotaxis Experiments Using 10% FCS as Chemoattractant

The migration behavior of cells is depended on the cell environment and on the chemoattractant being used. The example data shown here outlines the differences in migration behavior of HT-1080 cells cultured on a 2D surface (ibiTreat) and embedded in a bovine or rat tail collagen type I gel (1.5 mg/ml). 10% FCS was used as chemoattractant for all culture conditions. Cell migration was analyzed between time point 6 hours and time point 18 hours to ensure homogeneous cell behavior, and at the same time also to prevent effects caused by nutrient deprivation at later time points.

Directed cell migration can be assumed if the following parameters are fulfilled with statistical relevance: 1) the $FMI^{\parallel}$ value of the chemotaxis experiment should be larger than the $FMI^{\perp}$ and the p-value should be $p<0.05$; 2) the $FMI^{\parallel}$ and the $FMI^{\perp}$ of each control experiment should be around zero and the p-value should be $p>0.05$.

Table 2 Comparison of the migration parameters of HT-1080 cells cultured on a 2D surface (ibiTreat) or embedded in either bovine or rat tail collagen type I gel. Cell migration was analyzed for 30 to 40 cells from time point 6 hours to time point 18 hours. The data of the migration parameters of the control experiments is not given here.

	2D (ibiTreat surface)	3D (bovine collagen gel)	3D (rat tail collagen gel)
$FMI^{\parallel}$	0.19	0.19	0.21
$FMI^{\perp}$	0.06	-0.005	0.08
Directness	0.27	0.26	0.38
Euclidean distance [µm]	176.35	173.74	45.61
Velocity [µm/min]	0.93	0.92	0.15
Rayleigh Test [p-value]	6.42e-07	5.76e-09	0.01

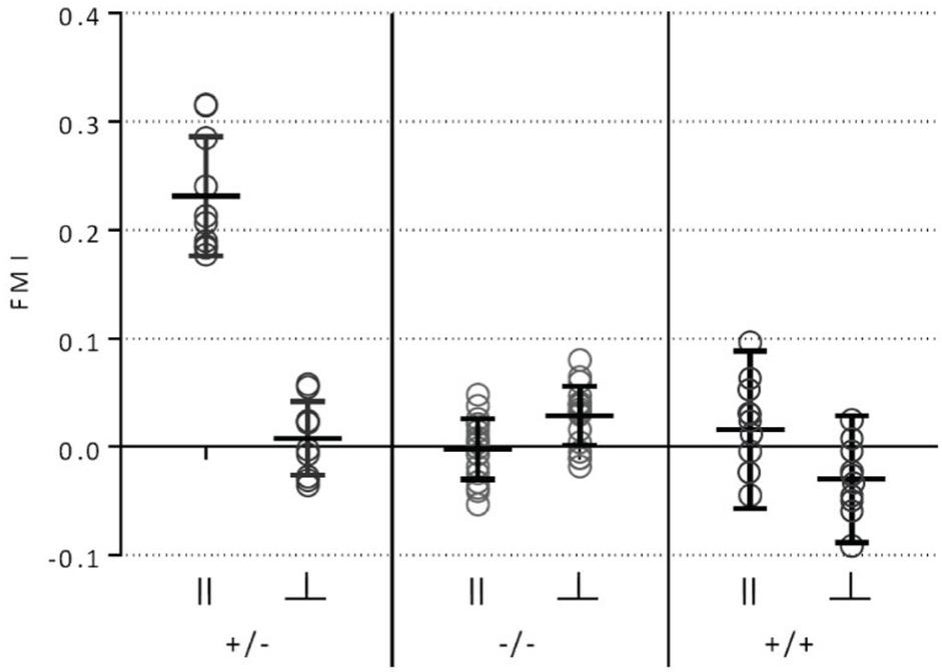


Figure 2 Comparison of the FMI values of 2D chemotaxis experiments and the corresponding controls. The data shown was obtained from HT1080 cells which were cultured on the ibiTreat surface and exposed to a FCS gradient. Directed migration can be assumed if the FMI^{II} value of the chemotaxis experiment is larger than the $FMI^{\perp}$ and if the FMI values of the control are around zero. Each dot represents one experiment.

A chemotactic response could be observed for all tested culture conditions. Both the FMI^{II} and the p-value (of the Rayleigh test) of the chemotaxis experiment indicate a directed cell migration. However, the migration parameter Euclidean distance and cell velocity differ significantly. Cells cultured on a 2D surface covered an average Euclidean distance of 176 µm with an average velocity of 0.9 µm/min. These finding could also be observed for cells embedded in 1.5 mg/ml bovine collagen type I gels. Contrary to this, a reduction of velocity of cells embedded in rat tail collagen gel resulted in an average covered distance of only 45 µm.

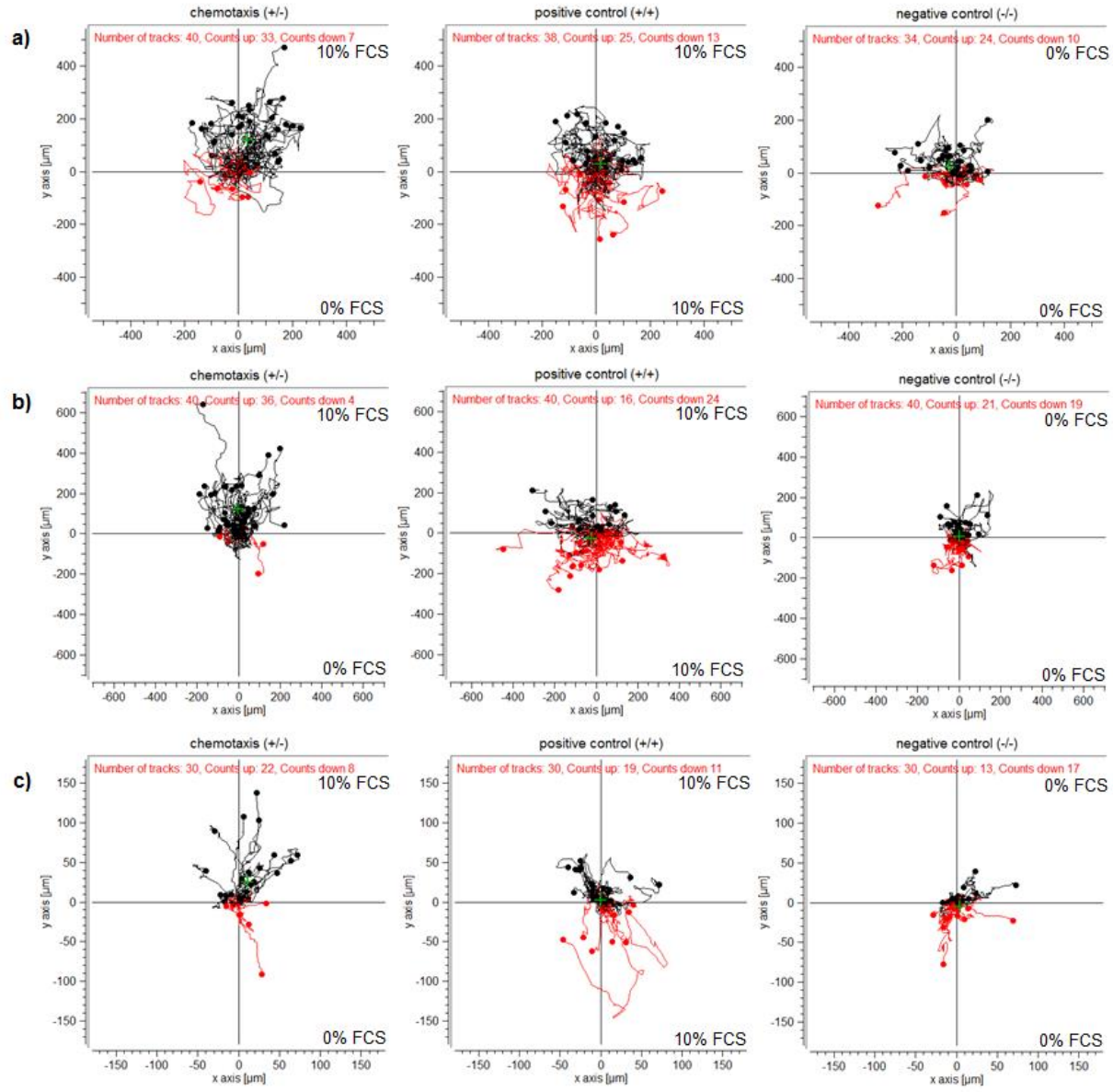


Figure 3 Representative cell trajectory plots of HT-1080 cells on a 2D surface (ibiTreat) (a), in a 1.5 mg/ml bovine collagen type I gel (b) and in a 1.5 mg/ml rat tail collagen type I gel (c). The results of a chemotaxis experiment (left), a positive (middle) and a negative control (right) are shown for each condition. The data shown represents the time range between time point 6 hours and 18 hours.

4.2. Inhibitory Effects of Latrunculin B

The latrunculins are a family of natural products and toxins produced by certain sponges. Latrunculins disrupt microfilament organization in cultured cells by binding to monomeric G-actin in a 1:1 complex at submicromolar concentrations. Latrunculin B (LatB) (Calbiochem, 428020) was used as a toxin in this study to test its effects on the chemotactical behaviour of HT-1080 cells.

A cell suspension was prepared in cell culture medium without any additives. Latrunculin B was added to the cell suspension in a final concentration of 0.2 µg/ml. Following an incubation time of 1 hour, the cells were embedded in a 1.5 mg/ml bovine collagen type I gel containing 0.2 µg/ml Latrunculin B and filled inside the µ-Slide Chemotaxis^{3D}. Additionally, a control experiment was performed in the same way, but in the absence of Latrunculin B. 10% FCS was used as chemoattractant for both experiments. Cell migration was analyzed between time point 6 hours and time point 18 hours to ensure homogeneous cell behavior, and at the same time also to prevent effects caused by nutrient deprivation at later time points.

The experimental data revealed that the presence of Latrunculin B has no effect on the directed migration of HT-1080 cells. However, it could be observed that the presence of Latrunculin B results in a decreased cell velocity.

Table 3 Comparison of the migration parameters of HT-1080 cells in the presence and the absence of Latrunculin B. Cell migration was analyzed for 30 to 40 cells from time point 6 hours to time point 18 hours.

	control experiment (+/-)	LatB (+/-)
$FMI^{\parallel}$	0.13	0.1
$FMI^{\perp}$	-0.02	0.068
Directness	0.28	0.29
Euclidean distance [µm]	125.96	101.48
Velocity [µm/min]	0.61	0.44
Rayleigh Test [p-value]	4e-03	0.02

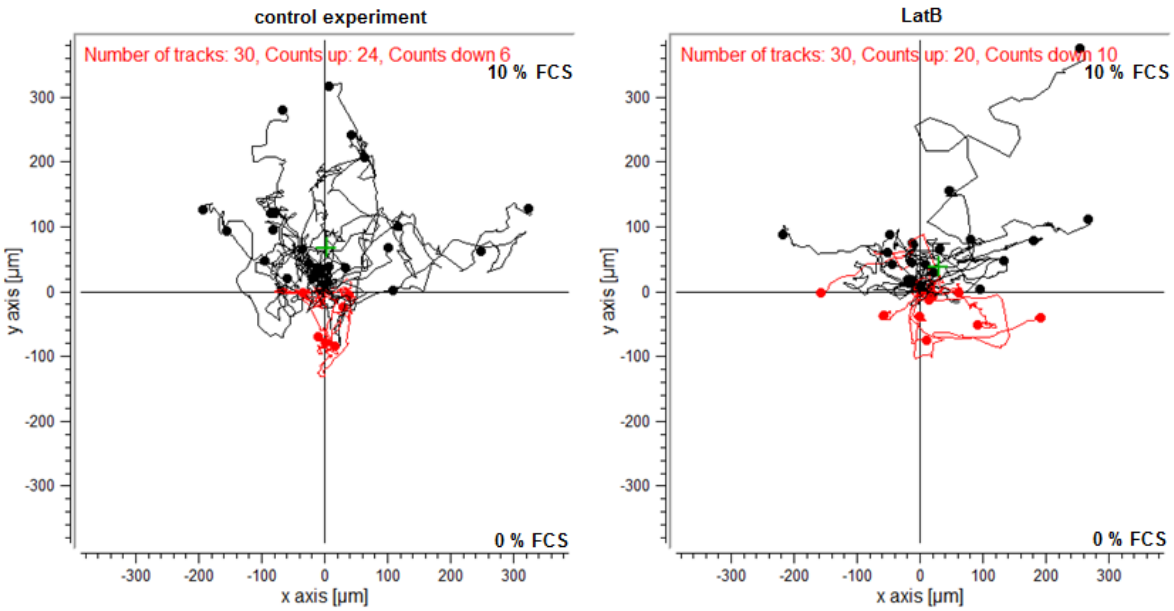


Figure 4 Trajectory plot of HT-1080 cells embedded in a bovine collagen type I gel. 10% FCS was used as chemoattractant. The left plot summarizes the cell track data of the control experiment, the right plot the data obtained in the presence of Latrunculin B. The data shown represents the time range between time point 6 hours and 18 hours.