Qualitative and quantitative analysis of slowly migrating chemotactic cancer cells in 3D

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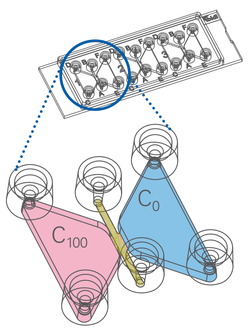
Abstract

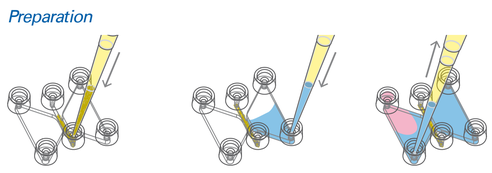
Chemotaxis of slowly migrating cells plays an important role in many physiological and pathological processes such as wound healing and cancer metastasis. While *in vitro* chemotaxis/migration assays of fast migrating cells such as dendritic cells are widely established, (live-cell) analysis of slowly migrating cells require a stable gradient over a relatively long period of time that is difficult to maintain.

We developed specialized chambers with confined geometries and materials that allow the investigation of slowly migrating cells along a chemotactic gradient that is stable over a period of 48h. Time-lapse phase-contrast microscopy, manual tracking and analysis of cell trajectories resulted in characteristic parameters to differentiate directed (chemotaxis) from undirected migration behavior. Our experiments revealed directed migration of HT1080 (fibrosarcoma) cancer cells in response to 10% fetal calf serum (FCS) and directed migration of MDA-MB-231 (human breast cancer) cells towards EGF in serum-free medium in a 3D Collagen gel.

A tool to study chemotaxis



Preparation

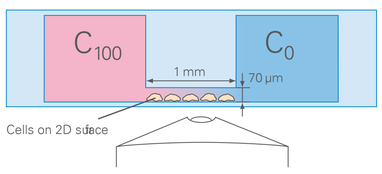


Seed cells with gel matrix — *Fill with chemo-attractant-free medium* — *Fill with chemoattractant*

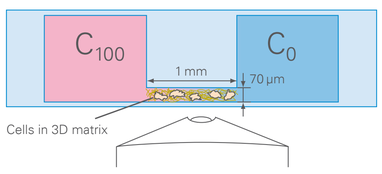
Basic Principle

Two large reservoirs of 60 µl are connected by a narrow observation area. The adherent cells or the cells embedded in a gel inside the observation area become super-imposed by a linear and time-stable gradient.

Chemotaxis on a 2D Surface

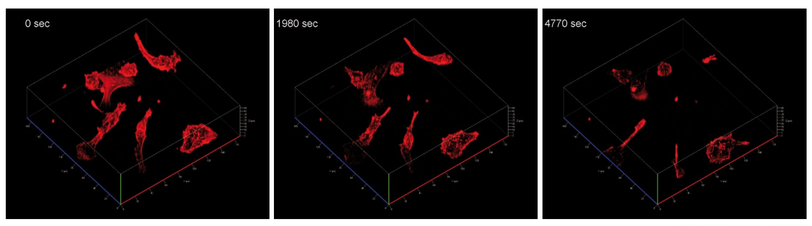


Chemotaxis in a 3D Gel Matrix



Microscopy slide to combine cell culturing and imaging
The µ-Slide Chemotaxis is designed to analyze chemotaxis of slowly migrating cells in 2D or 3D with real-time imaging techniques. The special geometry allows formation of a chemical gradient that is stable for up to 48h.

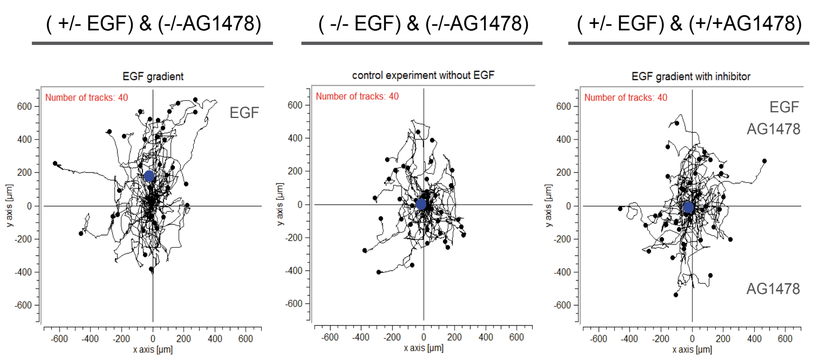
3D chemotactic migration of HT1080 cells



HT1080 LifeAct-RFP

3D migration of HT1080 cells in µ-Slide Chemotaxis
Confocal time-lapse microscopy of HT1080 cells expressing LifeAct-RFP in Collagen I gel reveals different migration behaviors dependent on substrate. Cells display ameboid-like movement on 2D surface, whereas cell shape is elongated when moving through Collagen I gel.

MDA-MB-231 cells migrate towards EGF



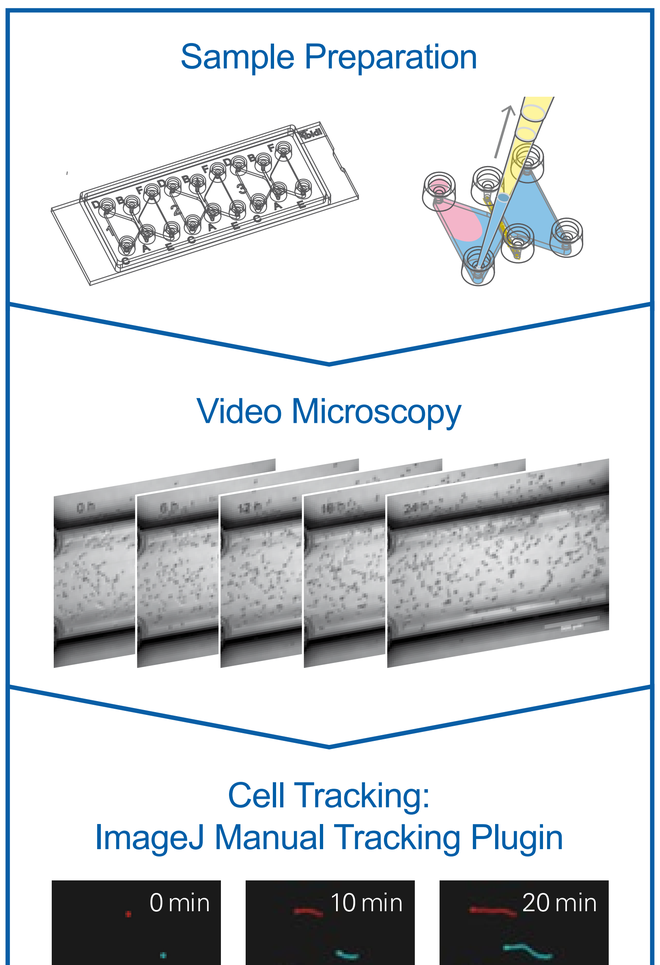
	-(+/- EGF) & (-/-AG1478)	-(/- EGF) & (-/-AG1478)	-(+- EGF) & (+/+AG1478)
FMI II	0.19	0.00	-0.03
Vel	0.69 µm/min	0.46 µm/min	0.55 µm/min
p	0.007	0.79	0.74

FMI ratio total distance: net distance;
p<0.05 Distribution is inhomogeneous, hence directed

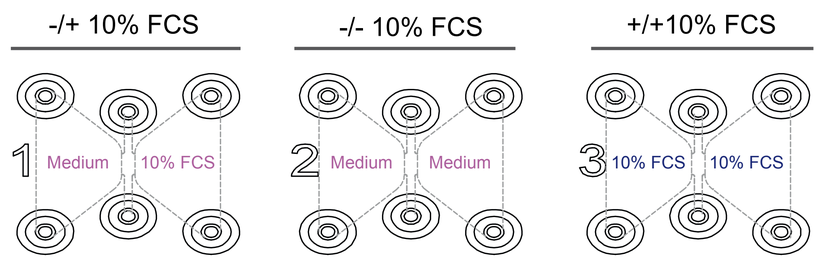
Selective directed migration towards EGF
MDA-MB-231 breast cancer cells show directed migration behavior towards EGF in serum-free medium.

Chemotaxis of HT1080 cells towards 10% FCS

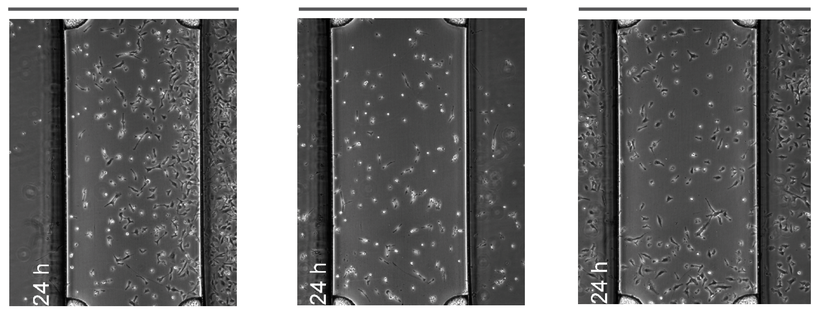
Workflow



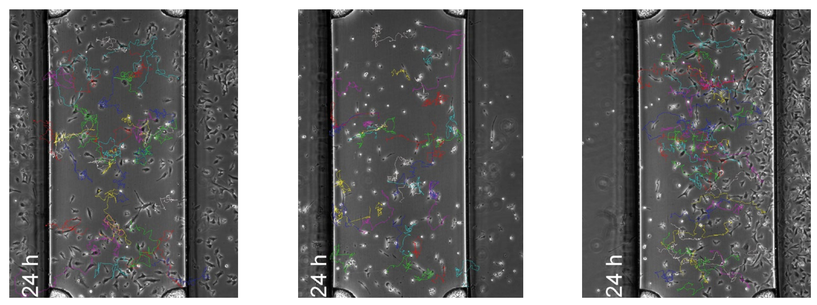
Experimental design



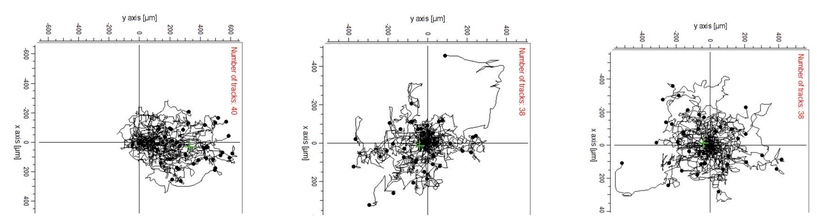
24h time-lapse, 10 min interval. 4x, phase contrast



Individual frame by frame tracking of 38 cells



Plotting individual cell tracks and normalization to same starting point



Parameter to characterize chemotactic migration

	-/+ 10% FCS	-/- 10% FCS	+/+ 10% FCS
FMI II	0.3	0.0	0.0
Vel	0.8µm/min	0.7µm/min	0.9µm/min
p	1.46E-13	0.351	0.302

FMI ratio total distance: net distance;
p<0.05 Distribution is inhomogeneous, hence directed

Workflow and chemotaxis experiment of HT1080 cancer cells towards FCS
Imaging, tracking and automated quantitative analysis of HT1080 cells reveals directed migration towards 10% FCS.