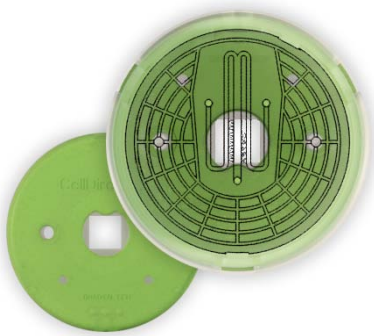
APPLICATION NOTE

GRADIENTECH

Live cell imaging of cancer cell chemotaxis in a stable gradient of hEGF

The effects of many chemotactic signaling proteins on cancer cell migration have not been thoroughly investigated. Cancer cells also often exhibit slow migration velocities. *In vitro* methods that enable generation of stable chemotactic gradients over extended periods of time are therefore advantageous.

CellDirector® 2D enables high-quality analysis of cell chemotaxis in response to stable gradients. Excellent cell culture conditions allow for time-lapse imaging of cell behavior for long periods of time with full gradient control.



Overview experimental steps	Estimated time per step
Cell culture prior to experimentation	According to the user's preferred protocols
1. Cell starvation and matrix coating	~ 15 min + 60 min incubation
2. Cell dissociation and seeding	~ 30 min + 60 min incubation
3. Starting the experiment	~ 10 min
4. Data collection by time-lapse microscopy	Typically 3 h
5. Data analysis	

CellDirector 2D overview

Two fluids are used for a CellDirector 2D experiment: the source fluid containing high levels of the substance to be examined (referred to as gradient substance), and the sink fluid containing low or no levels of the substance (Figure 1). Gradients are formed by diffusion of the substance of interest in the centrally positioned **gradient region**. The assay in addition features **two control regions** where cells experience no gradient, but instead are exposed to either the highest or lowest dose of the gradient substance.

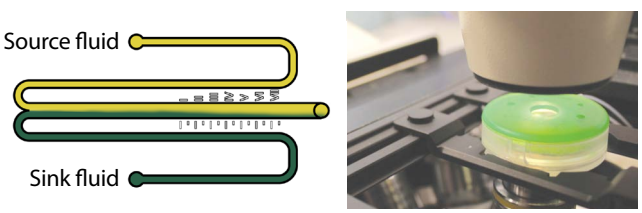


Figure 1. *Left panel:* Source and sink fluids form a steady-state gradient inside CellDirector 2D. *Right panel:* Analysis of cell migration in CellDirector 2D using a standard inverted microscope.

Cell culture and preparation of cells

Any adherent cell line can be used together with CellDirector 2D, and there is no need to develop new protocols for cell culture.

This application example shows the analysis of chemotaxis exhibited by human MDA-MB-231 breast cancer cells (Ref. 1) in response to a gradient of human epidermal growth factor (hEGF). Prior to chemotaxis analysis, the cells were grown according to the supplier's instructions (Ref. 2), and starved in serum free medium for 1 h.

Matrix coating and cell seeding

CellDirector 2D can be coated with an extracellular matrix (for example gelatin, collagen I or Matrigel) prior to cell seeding. Standard coating protocols apply. The matrix solution is infused in a one-step procedure into CellDirector 2D. Cells are seeded, again in a one-step procedure by pipetting cells into the single outlet tube of the assay.

In the current example, the MDA-MB-231 cells (5×10^5 cells/ml) were seeded onto a gelatin matrix and allowed to adhere for 1 h before starting the gradient experiment.

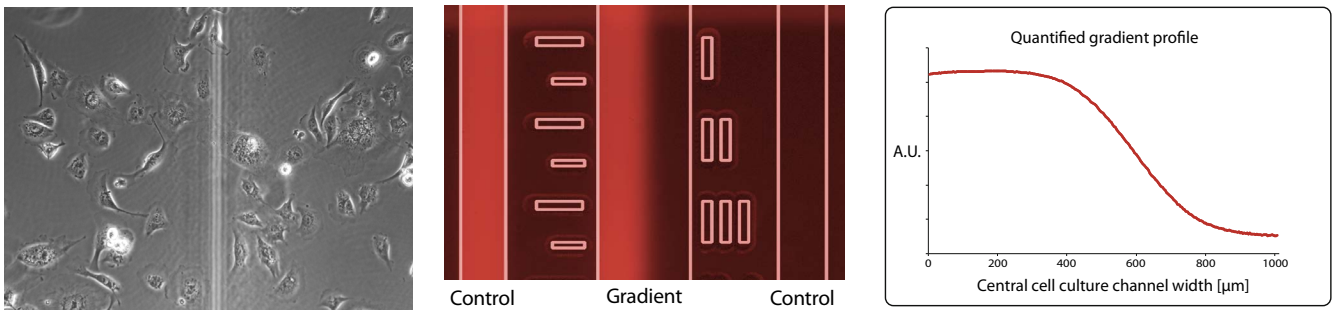


Figure 2. *Left panel:* Bright-field image of MDA-MB-231 cells growing in a steady-state gradient of hEGF inside CellDirector 2D. *Middle panel:* The gradient profile visualised by inclusion of Gradient Marker TRITC 20K (Gradientech) having a similar diffusion coefficient as hEGF. *Right panel:* Recorded gradient profile.

System assembly

After cell seeding, CellDirector 2D was placed in an inverted microscope fitted with a cell culture incubator for temperature control (Figure 1). CellDirector 2D was then connected to a syringe pump (Fusion 100, Gradientech) and a vacuum pump (Vacuum 104, Gradientech) to eliminate the risk of bubble formation during the experiment. The source fluid contained hEGF (50 ng/ml) and the sink fluid contained no hEGF. A stable gradient of hEFG was generated instantaneously by starting the syringe pump (Figure 2).

Data collection by time-lapse microscopy

Time-lapse imaging was performed by collecting bright-field images every 5 min over a period of 3 h. Images were collected from positions in the central cell culture channel (where the gradient is formed) as well as from the control channels.

Data analysis

Individual MDA-MB-231 cells were tracked and their migration paths plotted (Figure 3). For tracking and statistical analysis of data, the time-lapse image data sets were imported into the free Gradientech Tracking Tool™ software (download installation file at www.gradientech.se).
This experiment clearly showed that MDA-MB-231 cells has the capacity to chemotax in response to a gradient of hEGF.

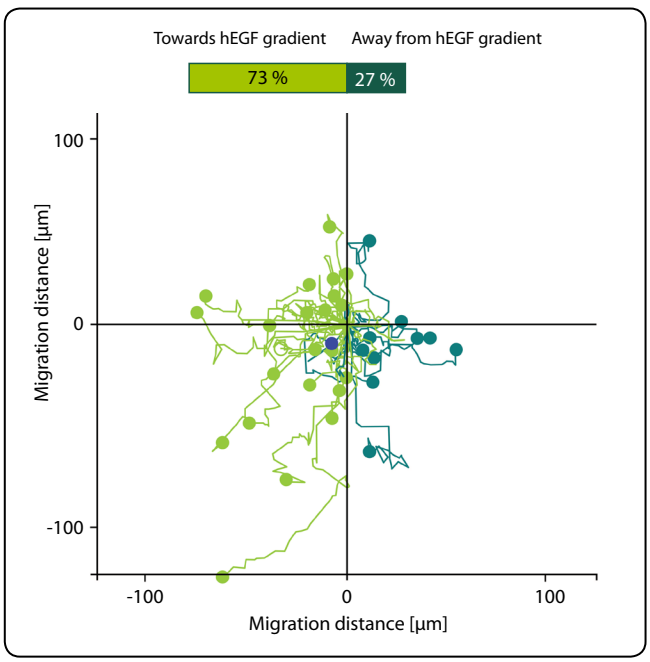


Figure 3. MDA-MB-231 breast cancer cells chemotax in response to a steady-state gradient of hEGF created inside CellDirector 2D.
73 % of all tracked cells were shown to migrate towards an increasing concentration of hEGF during a 3 h experiment. Data from control channels showed that cell migration is random in the absence of the hEGF gradient (not shown here).

Product information

PRODUCT	SUITABLE CELLS	APPLICATIONS	CATALOGUE #	SIZE
CellDirector® 2D	All types of adherent cells	Chemotaxis analysis of for example cancer cells, endothelial cells, neutrophils or neural cells.	REF 11-001-10	10 assays/box

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REFERENCES
1. Wang, S. et al. Differential effects of EGF gradient profiles on MDA-MB-231 breast cancer cells chemotaxis, Experimental Cell Research, 300, 180-189 (2004)
2. MDA-MB-231/GFP Cell Line, Product Data Sheet AKR-201, Cell Biolabs (2011)