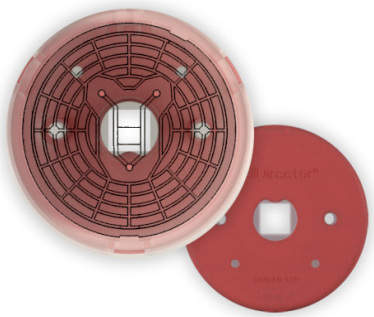
APPLICATION NOTE

gradientech

Directed cell migration from a single spheroid culture in a small-molecule gradient



The growth and migration of cancer cells in response to molecules is of importance for therapeutic studies. Single cancer spheroid cultures can be used for metastatic modeling, adding an extra layer of physiological complexity.

CellDirector 3D enables the study of single spheroid cultures in response to stable chemotactic gradients. Excellent culture conditions allow for live imaging of cell behavior over long periods of time with full gradient control.

Overview of experimental steps	Estimated time per step
1. Produce spheroids in hanging drop culture	According to the user's protocol
2. Degas the blister package in a vacuum chamber	30 min
3. Load single spheroid-matrix mixture into CellDirector 3D	10 min + 60 min polymerisation
4. Start the experiment	10 min
5. Collect live imaging data by time-lapse microscopy	72 h
6. Fix and stain spheroid culture	24 h

CellDirector 3D overview

Precise fluid flows in CellDirector 3D generate **stable gradients in a 3D matrix**. The biological material, in this case a single colorectal cancer spheroid, is suspended in an extracellular matrix of choice inside CellDirector 3D. A linear concentration gradient is formed by diffusion through the matrix. The loaded cells experience no shear force.

Once the steady-state gradient has been established, the **gradient shape is maintained during the whole experiment**. Cancer cell migration can be directly visualised and analysed using live imaging.

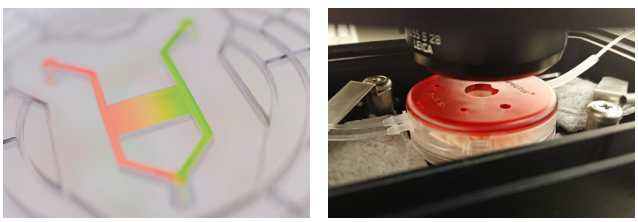


Figure 1. *Left panel*: A stable small-molecule gradient is formed by diffusion through the polymerised spheroid-matrix mixture inside CellDirector 3D. *Right panel*: CellDirector 3D set-up in a standard inverted microscope with and incubator stage for temperature and humidity control.

Preparation of spheroids in hanging drops

Prepare a single-cell suspension in phenol red-free DMEM ($6.25x10^4$ cells/ml). Pipette 40 µl on the lid of a plastic petri dish for a count of 250 cells per drop. Fill the petri dish with PBS and incubate the hanging drops for 2-3 days at 37°C. Note that spheroid preparation can differ among cell types [1].

Degassing and loading spheroid-matrix mixture

To avoid disturbances caused by growing bubbles in long experiments run at 37°C, degas the unopened blister package in a vacuum chamber for 30 min. Then, open the blister package inside a laminar flow hood and take out the CellDirector 3D assay.

Spheroids of 200 µm are ideal for working with CellDirector 3D. Remove 30 µl of media from a drop containing the spheroid and add 15 µl of collagen I matrix. Resuspend the spheroid pipetting lightly. Reverse pipette 9 µl of spheroid-matrix mixture into the CellDirector 3D chamber through the insert slit. If needed, tilt the chamber vertically to position the spheroid in the center region. Allow the matrix to polymerise for 1 h at room temperature [2].

Start the experiment and apply gradient

Fill one syringe with DMEM media only and the other one with DMEM containing the substance of interest, in this case a small molecule. Attach the syringes to an external syringe pump (Fusion 100, Gradientech) and connect the small vacuum pump (Vacuum 104, Gradientech) to the CellDirector 3D to minimize bubble formation during the experiment. Set the flow rate at 0.5 µl/min and make sure that the liquid exits both syringes before firmly inserting them into CellDirector 3D. With small molecules, a stable gradient is achieved after about 15 min.

Data collection by time-lapse microscopy

Place the CellDirector 3D in a microscope fitted with an incubation stage to ensure optimal cell culture conditions. Before starting a time-lapse, it is recommended to capture a tilescan of the entire chamber. Z-stack images (4 µm stepping) of the single spheroid were collected with a 20X dry objective every 2 h over a period of 72 h using an inverted microscope (Leica SP8).

Image analysis and data interpretation

Time-lapse images of cancer cells stained with CellTracker BOPIDY™ (Fig. 2) were used for image analysis. Movement of cancer cells within the spheroid was determined using the automated function of Gradientech Tracking Tool™ PRO software (Fig. 3). Preliminary results show that 55% of cells moved towards the gradient source while 45% moved in the opposite direction.

The distance and direction of migration away from the spheroid were determined from the Hoechst-stained spheroid (Fig. 2), using the center of the spheroid as the relative origin point. Preliminary findings point to greater cell migration towards the gradient source (data not shown).

Fixing and staining of spheroids

The protocol "*Immunostaining of samples within CellDirector 3D*" was used. Briefly, fix the spheroids for 10 min with 4% paraformaldehyde. Apply Hoechst solution (9 µl, 2 µg/ml) and incubate for 30 min. Place CellDirector 3D in PBS and shake at 4°C overnight. Dry and image the next day.

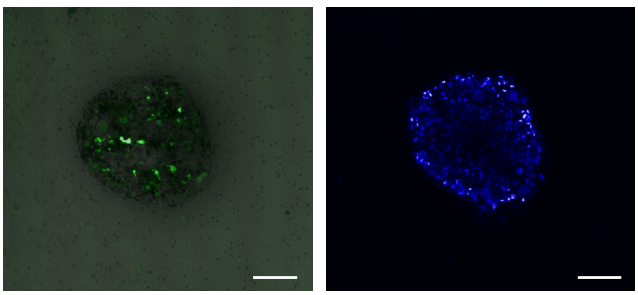


Figure 2. Representative example of field of view of a cancer spheroid inside CellDirector 3D. *Left panel:* Overlay of a bright-field image of a colorectal cancer spheroid stained with CellTracker BOPIDY™ (green). *Right panel:* Colorectal cancer spheroid fixed and stained with Hoechst. Scale bars: 100µm.

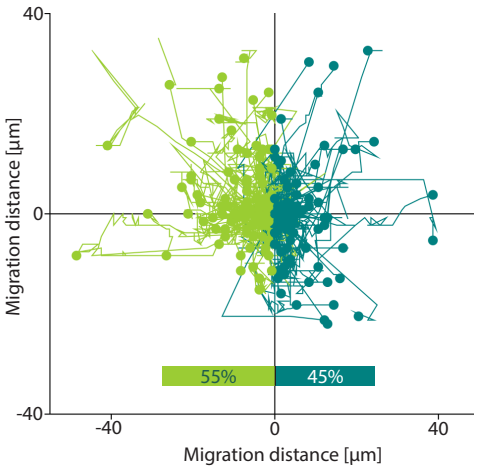


Figure 3. The polar plot shows cell movement within a single spheroid exposed to a small-molecule gradient (N=1). 55% of the cells were shown to migrate towards the gradient source in a 72 h experiment.

Product information

PRODUCT	SUITABLE CELLS	APPLICATIONS	CATALOGUE #	SIZE
CellDirector® 3D	Adherent and non-adherent cells	Chemotaxis and morphogenesis experiments in 3D matrix of single cells or more complex tissues	REF 10-001-10	10 assays/box

This Gradientech product is for research use only.

To request a quote or to order products from Gradientech
E-mail: sales@gradientech.se
Phone: +46 18 418 67 00
Fax: +46 18 418 67 01
or find your local distributor at http://www.gradientech.se

We thank Tia Tidwell and Hanne R. Hagland at the Centre for Organelle Research (CORE), Dept. of Mathematics and Natural Sciences, University of Stavanger, Norway, for sharing the protocol. This preliminary protocol is to be used only as guidance and is subject to change.

REFERENCES

[1] Kelm, JM. et al., *Method generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types*, Biotechnology and Bioengineering (2003).
[2] Kopanska, KS. et al., *Tensile forces originating from cancer spheroids facilitate tumor invasion*, PLOS ONE (2016).