GRADIENTECH

PROTOCOL
HeLa cell chemotaxis towards a gradient of FBS using CellDirector® 2D

CELLS AND REAGENTS NEEDED:
- HeLa cells (ATCC®-CCL-2™)
- Trypsin-EDTA 0.05% solution
- Dulbecco's modified Eagle's medium (DMEM)
- Fetal Bovine Serum (FBS)
- Phosphate Buffered Saline (PBS)

Preparation of HeLa cells

1. Culture HeLa cells in DMEM medium supplemented with 10% FBS at 37°C in a humid atmosphere with 5% CO_2.
2. Trypsinise the HeLa cells (5-10 min), than deactivate the trypsin with DMEM medium supplemented with 10% FBS.
3. Count and transfer the HeLa cells to a new tube.
4. Re-suspend the HeLa cells in DMEM medium supplemented with 10% FBS to a final concentration of 1x10^6 cells/ml.

Load HeLa cells into CellDirector® 2D and induce chemotaxis towards FBS

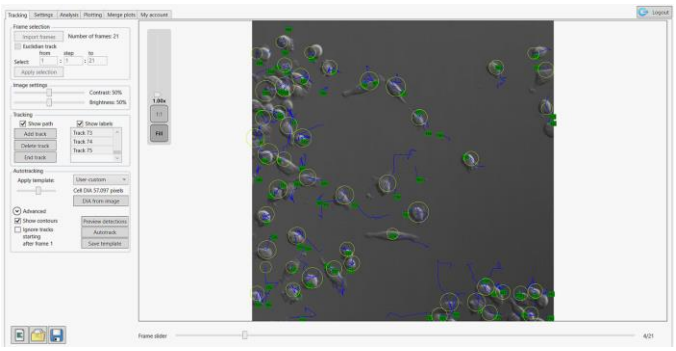
1. Pipet 200 µl HeLa cell suspension into an empty CellDirector® 2D.
2. Let the HeLa cells adhere overnight by placing the CellDirector® 2D assay, with the green side facing up, in a humidified incubator.

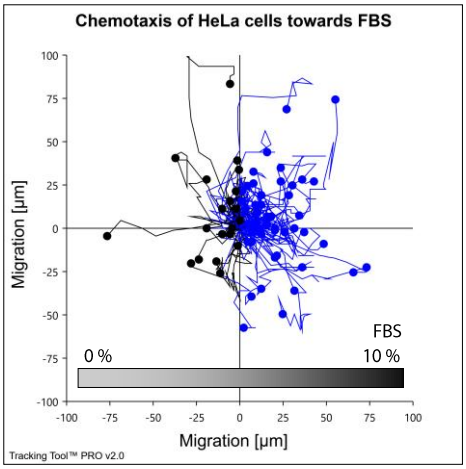
   NOTE: Overnight incubation will need the CellDirector® assay to be incubated in high humidity not to dry out. Preferably place the assay in a petri dish containing a wet tissue.

3. Change to starvation medium by slowly pipetting 200µL DMEM medium (no FBS) into the assay.
4. After 3-4 hours of starvation, prepare the syringes by filling one syringe with DMEM medium and the second syringe with DMEM medium containing 10% FBS.
5. Start the CellDirector® 2D experiment as described in the *Short User Guide*. See also the *Guide for Flow Directions and Gradient Orientation*.
6. Collect time-lapse images using bright-field microscopy (10x objective) at 0.2 fpm (1 image every 5 min). Preferably, collect images at serial regions of interest (ROI) along the gradient channel, as well as from the two control channels.
7. Continue collecting images for at least 2 hours (24 cycles).
8. Analyse using the automated cell tracking features in Tracking Tool™ PRO software (www.gradientech.se).

RESULTS:

Total number of tracked cells	66
Cells migrating towards increasing FBS	74%
Average velocity [µm/min]	1.3
Directionality	0.42





We thank Dr Maite Vidal-Quadras at the Dep. of Medical Biochemistry and Biophysics, Umeå University, Sweden, for sharing the protocol and the results.