

PROTOCOL

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

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

Preparation of HeLa cells

1. Culture HeLa cells in DMEM medium supplemented with 10% FBS at 37°C in a humid atmosphere with 5% CO₂.
2. Trypsinise the HeLa cells (5-10 min), then 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 1x10⁶ cells/ml.

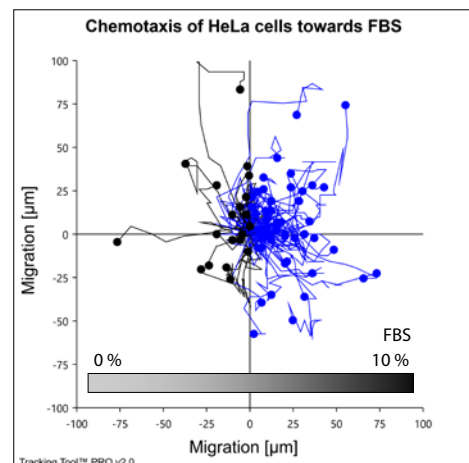
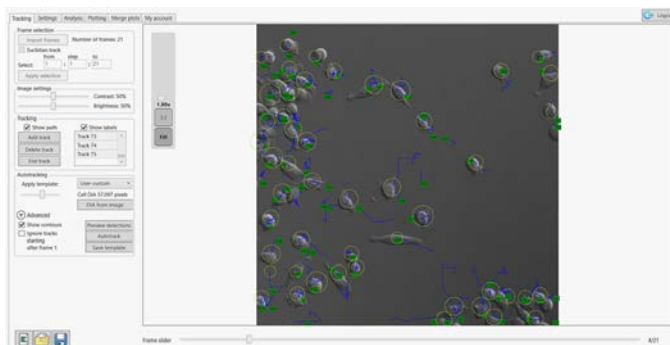
Load HeLa cells into CellDirector® 2D and induce chemotaxis towards PDGF-B

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 PDGF-B 74%
 Average velocity [µm/min] 1.3
 Directionality 0.42



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