GRADIENTECH

PROTOCOL
Human Neutrophil chemotaxis towards a gradient of δ-decalactone using CellDirector® 2D

CELLS AND REAGENTS: Human Polymorphonuclear Neutrophils (PMNs)
δ-decalactone
RPMI 1640

Preparation of human PMNs

1. Isolate human neutrophils from buffy coat samples or whole blood.
2. Adjust the cells to a concentration of $1x10^6$ /mL using RPMI 1640.

Cell loading of CellDirector® 2D

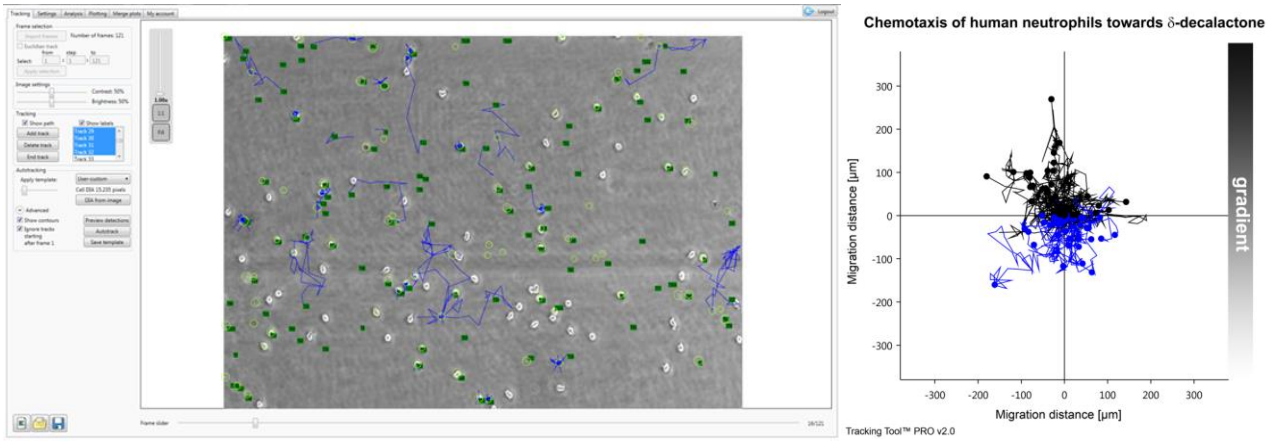
3. Pipet 200 µL of the cell suspension slowly into an empty CellDirector 2D.
4. Incubate the cell chamber at 37°C and 5% CO_2 for 1–2 h.

Induce chemotaxis towards δ-decalactone

5. Connect the CellDirector 2D chemotaxis chamber to a vacuum pump and to two syringes, containing the chemoattractant and the vehicle control, respectively.
6. Place the syringes in a syringe pump.
7. Set the flow rate to 1µL/min.
8. Collect images using bright field microscopy (10x objective) every 30 s for 1 h from the gradient position as well the two control positions.
9. Analyze migration data using the Tracking Tool PRO v2.0 software (www.gradientech.se).

RESULTS:

Total number of tracked cells	107
Cells migrating towards increasing δ-decalactone	57%
Center of mass (y) [µm]	1.5



We thank Dr. Gaby Andersen at Deutsche Forschungsanstalt für Lebensmittelchemie - Leibniz Institut, Freising, Germany for sharing the protocol and the results.

Version 20160630