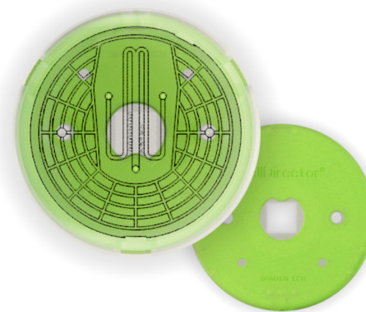
Chemotaxis of mouse neutrophils in response to a MIP-2 gradient

Neutrophils are the most abundant type of white blood cell, and they help to defend the body against infections. This application note shows an example of how CellDirector® 2D can be used to study and firmly establish how neutrophils chemotax towards a defined and stable concentration gradient of Macrophage Inflammatory Protein-2, MIP-2.



Setting up a chemotaxis experiment using CellDirector® 2D is a quick and easy procedure, something that is of great importance when studying short-lived and fast-responding cells like neutrophils.

Overview of experimental steps	Estimated time per step
1. Neutrophil extraction	According to user protocol
2. Neutrophil activation	5 min
3. Neutrophil seeding	1 h
4. Starting the experiment	10 min
5. Data collection by time-lapse microscopy	30 min
6. Data analysis	1 h

CellDirector® 2D overview

Two different cell culture media solutions are used for each CellDirector 2D experiment. The source medium contains a high concentration of the chemotactic substance to be evaluated, whereas the sink medium contains less or nothing of the chemotactic substance (Figure 1). Gradients are formed in the centrally positioned **gradient region** by diffusion of the chemotactic substance between fluid streams generated from the two different media solutions. Importantly, CellDirector 2D also features **two internal control regions** where cells experience no gradient, but are instead exposed only to the source or the sink media solution.

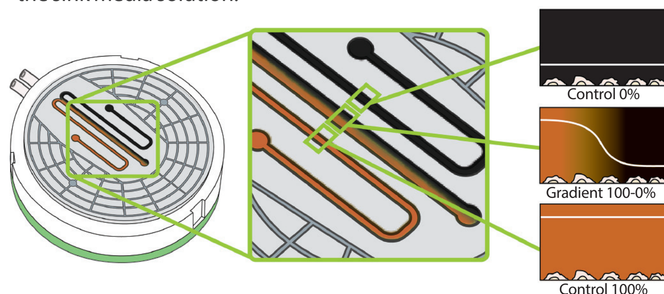


Figure 1. Stable and well-established gradients of soluble chemotactic factors are formed in the centrally positioned channel in CellDirector 2D.

Neutrophil extraction and activation

In the present example, neutrophils were initially isolated (Ref. 1) from mouse bone marrow according to a well-established protocol (similar to protocol in Ref. 2). The neutrophils were pre-activated by resuspending them in 50 ng/ml MIP-2 in RPMI-1640 and left for incubation for 5 min at 37 °C. The neutrophil preparation was next centrifuged and diluted to a final concentration of 0.25×10^6 cells/ml in RPMI-1640.

Neutrophil seeding

200 µl of the neutrophil suspension was seeded directly into CellDirector 2D via the single outlet tube.

No matrix coating of the cell culture channels was needed as the neutrophils adhered readily to the glass substrate.

CellDirector 2D was incubated at 37 °C for 1 h.

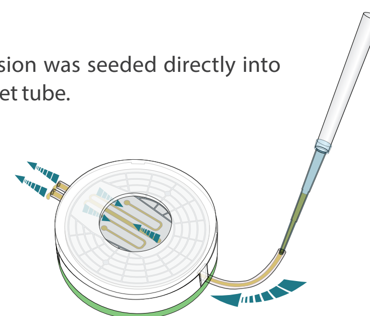
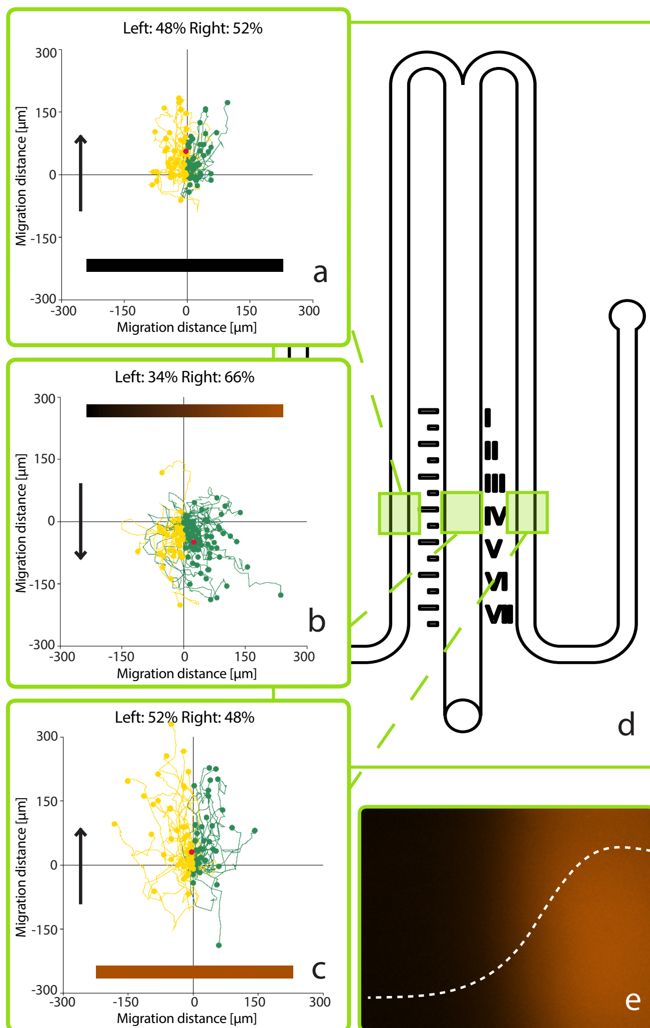


Figure 2. Matrix coating, as well as loading of cells, is easily achieved by manual pipetting into the single outlet tube.



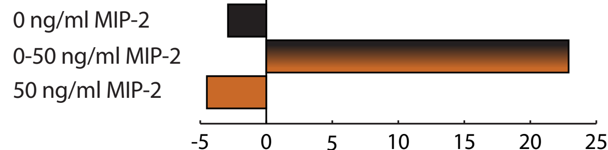


Figure 4. The average displacement (center of mass) of neutrophils towards a higher concentration of MIP-2 during during a 40 min chemotaxis experiment.

Starting the experiment

CellDirector 2D was connected to two syringes filled with either the source medium (RPMI-1640 containing 50 ng/ml MIP-2) and Gradient Marker TRITC 20K for gradient visualisation, or with the sink medium (RPMI-1640 only). CellDirector 2D was then placed under a microscope fitted with a cell incubator set to 37 °C at 5% CO_2.

Data collection by time-lapse microscopy

Time-lapse imaging was used to collect images from the gradient channel as well as from the two internal control channels. Brightfield images were collected every minute for 2 h due to high neutrophil migration velocities.

Data analysis

Cells in the central gradient channel as well as in the two control channels were tracked and analysed using Gradientech's Tracking Tool™ PRO software (free to download at www.gradientech.se). The plots clearly show that a gradient of MIP-2 ranging from 0-50 ng/ml very potently induces chemotaxis of mouse neutrophils towards higher levels of MIP-2.

Figure 3. a) Control channel with 0 ng/ml MIP-2. b) Gradient channel with 0-50 ng/ml MIP-2 with increasing concentration to the right. c) Control channel with 50 ng/ml MIP-2. Black arrows show the direction of fluid flow. d) Positions where images were collected. e) Image of Gradient Marker TRITC in the gradient channel. The dashed line represents the quantified gradient.

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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To request a quote or to order products from Gradientech
Email: sales@gradientech.se
Phone: +46 18 418 67 00
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REFERENCES
1. Neutrophils were isolated in collaboration with the Mia Phillipson Group, Dept. of Medical Cell Biology, Uppsala University, Sweden.
2. Heit, B. et al. PI3K accelerates, but is not required for, neutrophil chemotaxis to fMLP, Journal of Cell Science, 121, 205-214 (2008)