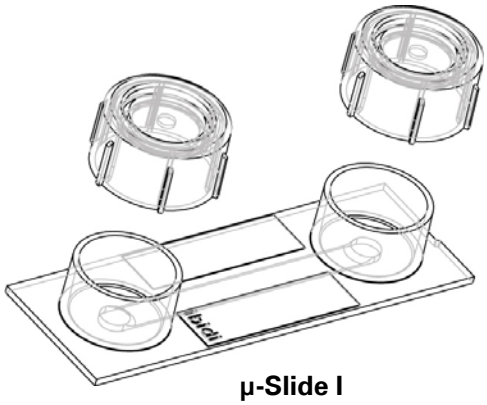
Concentration gradients inside µ-Slide I

The microfluidic channel of the **µ-Slide I** is suited to set up chemical gradients. By a simple pipetting procedure a concentration profile can be established. The profile is well defined and has been shown to stimulate chemotaxis in fast migrating cells such as *Dictyostelium discoideum*.
In contrast, for chemotaxis experiments of slow migrating cells and cells in a 3D matrix we recommend the µ-Slide Chemotaxis 2D (80306) and µ-Slide Chemotaxis 3D (80326), respectively.

Short Protocol

- Seed cells into µ-Slide I and wait until cells have fully attached. See step **"1. Seeding cells"**.
- Establish the gradient following step **"2. Pipetting procedure"**.
- Find the steepest point of the gradient following procedure **"3. Determination of observation area".**
- Analyze cell movement by time-lapse microscopy.



µ-Slide I

1. Seeding cells

- Prepare cells as usual and fill 100 µl of the cell suspension into the channel (see **Fig. 1** A). We recommend 3-7 x 10^5 cells/ml.
- Cover reservoirs loosely with the supplied caps. Await cell attachment.
- For longer cultivation fill each reservoir with 600 µl cell-free medium.
- For more details on cell seeding see the instructions for µ-Slide I or visit www.ibidi.com.

2. Pipetting procedure for the gradient

To set up the concentration profile you only need a standard laboratory pipette and your chemoattractant solution.

- Empty the reservoirs if necessary. Leave the channel filled with 100 µl of medium (without chemoattractant).
- Apply 20 - 40 µl of your chemoattractant in one reservoir (see **Fig. 1** B)
- Aspirate the same amount of liquid (20 - 40 µl) from the opposite reservoir (see **Fig. 1** C).
- Cover reservoirs with supplied lids.

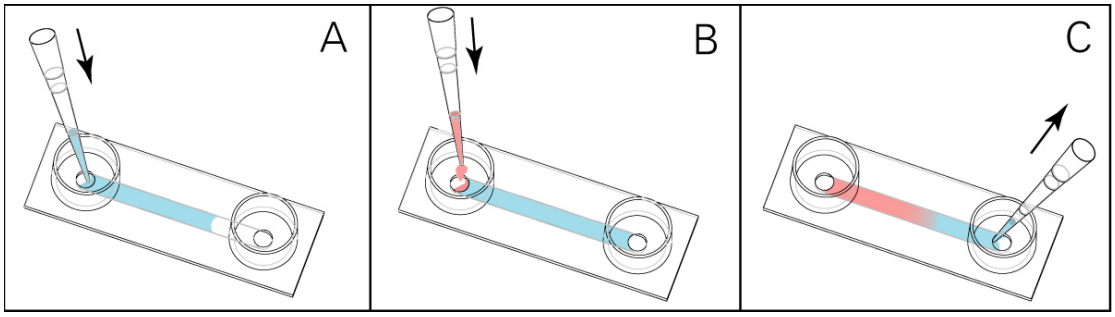


Fig. 1 Pipetting the gradient

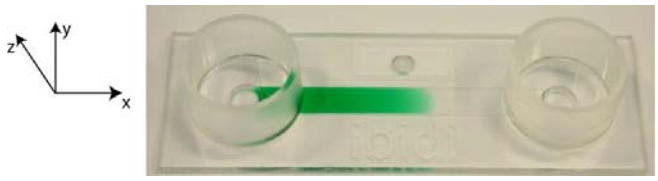


Fig. 2: Image of gradient visualized by food coloring. Make sure the colored solutions are identical in density. If not, the colored and non-colored liquid may flow into each other in a non-defined way.

3. Determination of observation area

Responses of cells depend on the area of observation. Using beads it is possible to visualize the chemoattractant gradient. We recommend the following procedure:

- Small microscopy beads of any kind can be used. We suggest e.g. for FITC filter sets fluorescent beads from Molecular Probes, (FluoSpheres®, 10 µm F8836). Please check for your desired fluorescence wavelength or use phase contrast microscopy.
- Recommended concentration of beads: 5 x 10^6 beads/ml.
- Add the beads directly to your chemoattractant solution and apply to the channel as described in section 2.
- Find the position of the bead which is rinsed farthest into the channel. For 40 µl chemoattractant solution the expected position is about 7-9 mm from the center of the channel (or 30 mm from the channel's aperture).

The steepest gradients can be found in a region near x_{max}. This area is at least 1.5 mm wide. To visualize the concentration profile while using cells we recommend the use of fluorescent beads to determine where the cells should be observed.

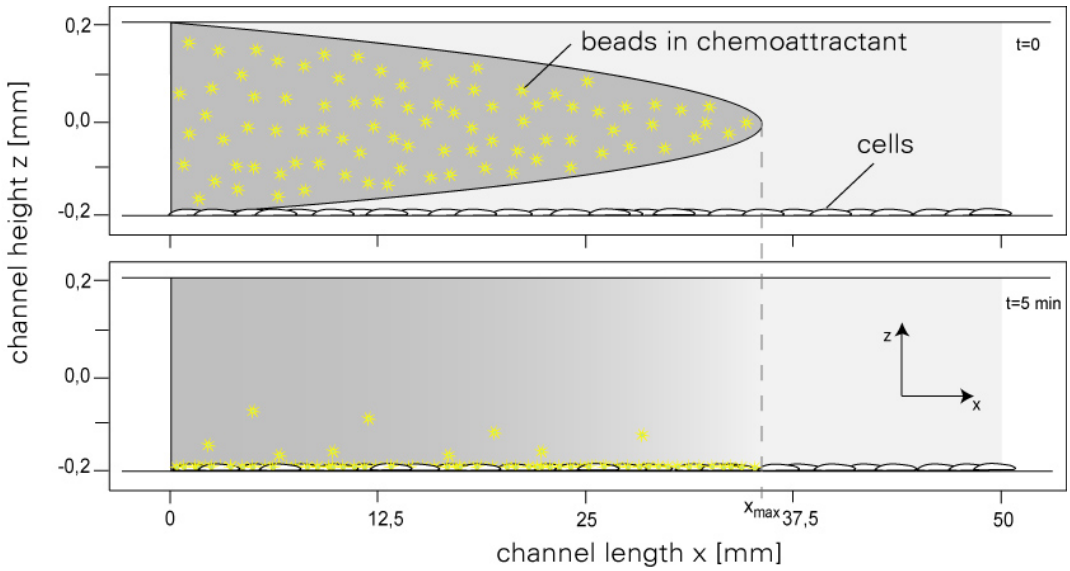


Fig. 3 After flushing chemoattractant and beads into the channel a stretched parabola-like shaped form is created. After some minutes the gradient is established and beads fall down showing the area of interest.

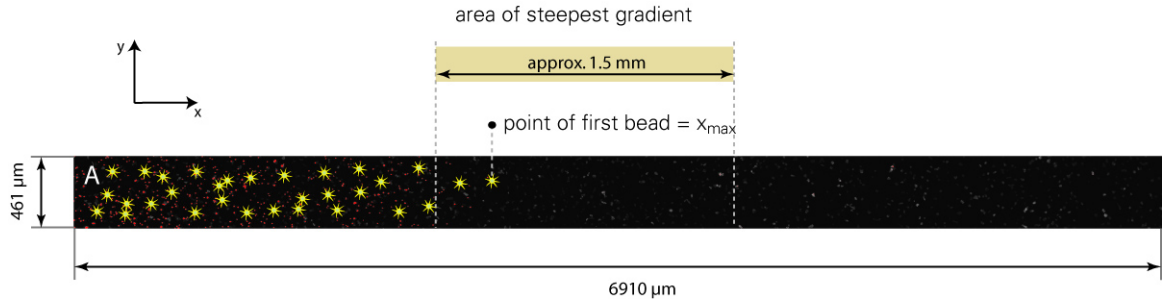


Fig. 4 The steepest gradient area is defined by the first bead inside the attractant solution.

4. Concentration profile

Measurements with the fluorescent dye Rhodamine 6G showed that the distribution of chemoattractant is half-parabola shaped (solutions 10 µM and 100 µM, 40 µl Rhodamine as chemoattractant).

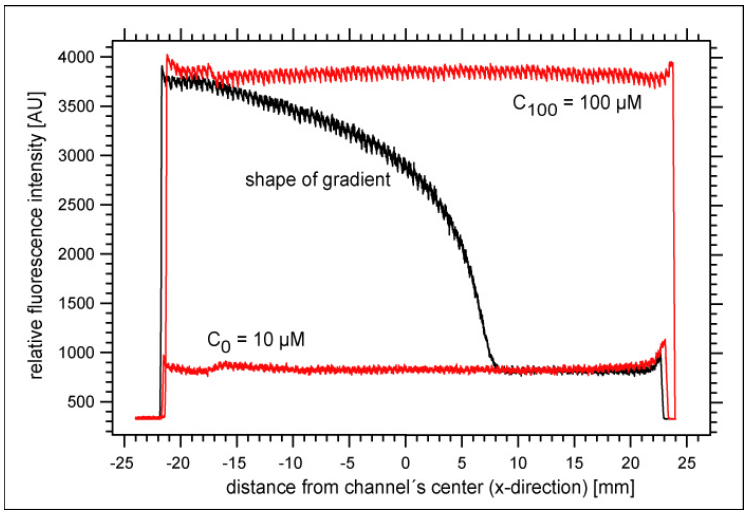


Fig. 5: Concentration profile measured with a fluorescence dye. The fluorescence intensity is plotted versus the distance in x direction. The two references C_0 and C_{100} were measured in separate channels

The shape of the concentration profile indicates that the profile is originated in the characteristic flow profile between two parallel plates. Measurements were taken 10 µm above the cells using confocal microscopy. 40 µl of chemoattractant solution was used. Different volumes of chemoattractant result in similar shapes.

5. Time development of the concentration profile

Dependent on diffusing chemoattractant, viscosity, and temperature the gradient will be blurred after some time.

Small chemoattractant molecules (e.g. cAMP):
The observation time starts immediately after the pipetting procedure. Small molecules will form a proper gradient for single cell applications for approx. 30-60 min.

Large chemoattractant molecules (e.g. VEGF):
The observation time starts immediately after the pipetting procedure. The gradient is steep enough for single cell chemotaxis for approx. 0.5-2 hrs.

Although the gradient is visible for much longer time it is strongly dependent on the experiment whether the gradient is steep enough for single cell chemotaxis. Thus, experiments dealing with different concentrations on different positions in one channel are possible for much longer times.

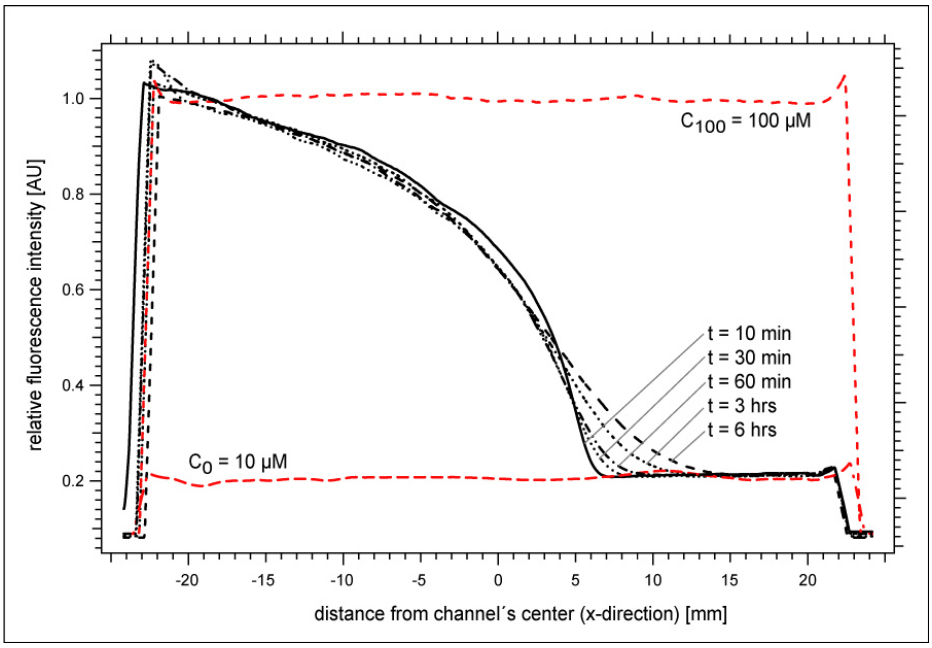


Fig. 6 The gradient flattens over time.

For more time stable gradients we recommend our µ-Slide Chemotaxis 2D and µ-Slide Chemotaxis 3D. Both provide much longer gradient stability (more than 48 hrs).

Please contact us for further information. (info@ibidi.com)