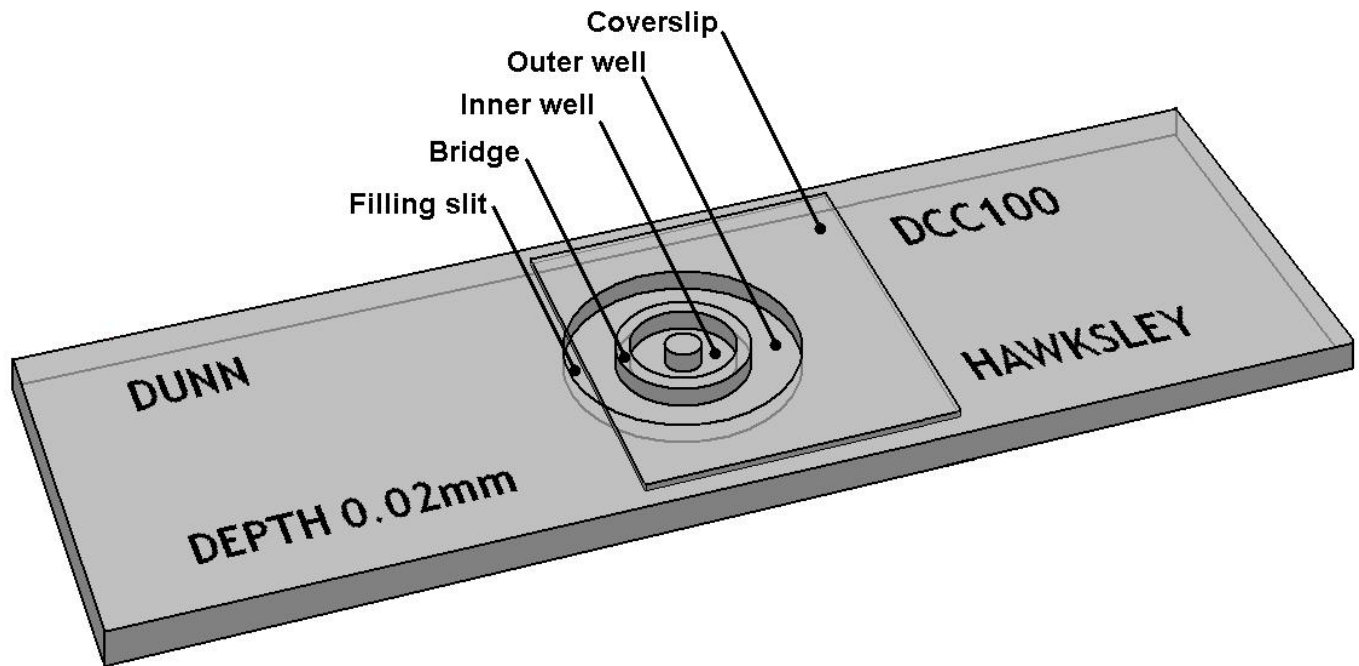


Using the Dunn Chemotaxis Chamber (Hawksley DCC100)

GRAHAM A. DUNN

Randall Division, New Hunt's House, King's College London, London, SE1 1UL, UK



Design

The Dunn Chemotaxis Chamber (DCC100) allows the behaviour of cells subjected to a linear concentration gradient of chemoattractant to be observed directly in the light microscope. The chamber was designed to have good optical properties and long-term stability of the gradient, thus permitting time-lapse recording of cell behaviour over many hours.

The chamber consists of a glass microscope slide with two concentric annular wells ground into the centre of one face to a depth of about half the thickness of the slide (see figure). The annular platform that separates the wells (the bridge) is about 1mm wide. This bridge and the central pip are optically polished to lie precisely 20 μm below the slide's face. Thus when the wells are covered with a coverslip carrying the cells to be studied, there is a gap between coverslip and bridge of 20 μm . If the inner well of the chamber is filled with control medium and the outer well filled with a medium containing chemoattractant, a radially directed linear diffusion gradient becomes quickly established in this gap and is subsequently maintained for several hours.

Setting up the chamber

Cells are seeded onto a suitably washed sterile coverslip and allowed to settle prior to assembling the chemotaxis chamber. Special thick coverslips are provided that ensure dimensional stability, though other types can be used with care. Initially, both annular wells are filled with control medium and the coverslip seeded with cells is inverted onto the chamber in an offset position in order to leave a narrow filling slit at one edge for access to the outer well (see figure). After firmly seating the coverslip on the face of the chamber and blotting up surplus medium, it is sealed in place using hot wax mixture (Vaseline : paraffin : beeswax - 1:1:1) applied with a paintbrush around all the edges except for the filling slit. Take care not to apply any pressure directly over the bridge or you will crush the cells! In order to set up a chemotactic gradient, the medium is then drained from the outer well using a syringe with a fine-bore needle and replaced with medium containing the chemoattractant. The slit is finally sealed with the hot wax mixture. If the chemoattractant is a globular protein of around 20 kDa, the gradient becomes linear within approximately 20 min and has a half-life of about 24 h (Zicha *et al.*, 1991). Large changes in molecular weight will have only a small effect on these times.

Recording cell behaviour

The chamber is placed on a temperature-controlled microscope stage with suitable provision for time-lapse recording of cell migration in the gradient. It is usually positioned by locating the outer edge of the bridge to coincide with the upper margin of the recording field, so that the direction of increasing chemoattractant concentration is vertically upwards in the image. The useful recording field is limited by the width of the bridge and can have a maximum size at the specimen of about 1 mm in the vertical direction. However, a motorised X-Y stage under software control will allow cell behaviour in several different fields over the bridge to be recorded during each time-lapse interval, thus maximising the data collected in a single experiment. Cells over the central pip region are not subjected to a gradient and simultaneous recording of their behaviour may be a useful control in some experiments. The design of the chemotaxis chamber, especially the blind inner well, helps to ensure stability of the gradient but it is advisable not to handle the chamber roughly during the course of an experiment.

Cell tracking and analysis of chemotaxis

The method of tracking cells in the recordings in order to obtain trajectories of cell locomotion will depend on the software available and specific details cannot be given here. Chemotaxis can be evaluated by assessing directional clustering of cell migration using standard methods for the statistical analysis of directional data (Zicha *et al.*, 1997).

References

- Zicha, D., Dunn, G.A. and Brown, A.F. (1991) A new direct-viewing chemotaxis chamber. *J. Cell Sci.* **99**, 769-775.
- Zicha D., Dunn G. and Jones G. (1997) Analyzing chemotaxis using the Dunn direct-viewing chamber. *Methods Mol. Biol.* **75**, 449-57.