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Measurement of cell migration in response to an evolving radial chemokine gradient triggered by a microvalve†

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Abstract

We describe a novel chemotaxis assay based on the microvalve-actuated release of a chemoattractant from a cell-free microchamber into a cell-containing microchamber. The microvalve chemotaxis device (μ VCD) was placed on the stage of a conventional inverted microscope to obtain time-lapse micrographs of neutrophils migrating in a radially-symmetric evolving gradient of the chemotactic factor CXCL8/Interleukin-8. A fluorescent tracer was added to the CXCL8 solution to visualize the evolution of the gradient profile, so that at each time point the cell positions could be assigned CXCL8 concentration values. Tracking of individual neutrophils for 90 minutes showed that (a) the neutrophil migratory response is, on average, radially directed towards the CXCL8 source; (b) significant non-radial displacements occur frequently; and (c) there is considerable heterogeneity in the migration speeds and directions amongst the neutrophil population. A custom-made imaging analysis tool was used to extract measurements of migratory behavior such as speed, velocity along the gradient's radial axis, and the cosine of the turning angle as a function of CXCL8 concentration. The μ VCD can be easily adapted to study the migratory behavior of cultured cells other than neutrophils.

Introduction

Leukocyte migration is essential for the innate immune response to microbial pathogens and immune system organization.¹⁻⁶ The migration of leukocytes from the vasculature into tissue involves a number of different molecules including adhesion molecules and chemoattractants.⁴ Chemokines, a family of endogenous chemotactic factors made up of small basic peptides (8–14 kDa),² are responsible for the directed migration of leukocytes from the bloodstream into tissue.^{1,6,7} CXCL8, a member of the CXC family of chemokines (historically known as Interleukin-8 or IL-8), is an important neutrophil chemotactic factor.^{1,8}

Neutrophil migration has been studied *in vitro* using endpoint assays such as Boyden chambers⁹⁻¹¹ (also known as “transwell assays”) and visual assays such as the classical “under-agarose” assay (reviewed in ref. 12), the Zigmond chamber,¹³ the Dunn chamber,¹⁴ and the classical micro-pipette-based assay.¹⁵⁻¹⁷ Whereas these traditional chemotaxis assays provide important information on the migratory responses of neutrophils (micropipette assays suffer from additional, severe throughput constraints), they do not

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allow for a precise, reproducible control over the temporal evolution of the gradient. A microfluidic gradient generator based on laminar flow has been developed for assaying neutrophil chemotaxis in response to static gradients perpendicular to the flow lines.^{18,19} The flow-based gradient generator is attractive because it provides quantitative knowledge of the chemotactic gradient. However, the exposure of cells to flow raises three concerns: (1) since cells use mechanical forces to migrate within the gradient, shear stress exerted by the flow may yield confounding results;²⁰ (2) *in vivo*, the neutrophils migrate towards point sources (*i.e.* macrophages at the site of inflammation), a geometry that cannot be replicated under continuous flow; and (3) the microfluidic gradient generator^{18,19} cannot easily reproduce the spatiotemporal decay of the concentration field expected from a point source. Beebe and colleagues have proposed a gradient generator based on diffusion through a membrane.²¹ Last but not least, all the above methods require a major human intervention (*i.e.* dispensing fluid or connecting an inlet) to establish the concentration field, which can result in a large uncertainty in the concentration values at early time points.

Here we describe a novel neutrophil chemotaxis assay based on a microfluidic device with microvalves (termed “microvalve chemotaxis device” or μ VCD) that generates a temporally-evolving, radially symmetric gradient of CXCL8; the gradient forms by diffusion only (in the absence of flow) and at a time point precisely specified by the user. We also demonstrate a custom-made image analysis tool that correlates temporally-evolving concentration fields with the position of the cells.

Experimental

Fabrication of the microfluidic device

The microvalve chemotaxis device (μ VCD) was fabricated entirely from the elastomer polydimethylsiloxane (PDMS) using standard soft lithography techniques.²² As shown schematically in Fig. 1, the μ VCD is assembled by stacking three PDMS layers; a similar microvalve design has been reported previously.^{23–25} Briefly, the top layer (forming the fluidic channels) and the bottom layer (forming the pneumatic lines) were both fabricated by molding PDMS against silicon masters patterned with SU8 photoresist (Fig. 1a) that defined complementary figures in the PDMS replica (Fig. 1b). The middle layer consists of an approximately 12 μ m-thick PDMS membrane fabricated by spin-coating and curing a thin layer of PDMS precursor solution onto a featureless silicon wafer. After fabrication of the separate layers, the membrane was irreversibly bonded to the control layer (Fig. 1c) and detached from the silicon wafer (Fig. 1d).

Silicone tubing inlets connecting to the fluidic layer were bonded to the device during the PDMS molding process.²⁶ To create inlet connections for the pneumatic lines, sections of the PDMS membrane bonded to the control layer were removed to uncover those photolithographically-defined inlet pads. Oxygen plasma was then used to bond a PDMS block (containing silicone tubing inlets aligned to these inlet regions) to the intact membrane surrounding the inlet regions. To bond the layers, we exposed them to oxygen plasma and applied suction to the pneumatic lines before aligning the layers and bringing them into contact, which irreversibly bonded them in a few seconds (Fig. 1e).

Chemotaxis assay

Before cells and CXCL8 were loaded into the μ VCD, the microchambers were coated with poly-D-lysine (10 μ g mL⁻¹) for 30 minutes at room temperature, followed by fibronectin (10 μ g mL⁻¹) for one hour at room temperature. Subsequently, the devices were flushed with Hanks Balanced Salt Solution (HBSS) with 0.1% wt. bovine serum albumin (BSA) prior to being used, in order to prevent non-specific binding of CXCL8 to the device surfaces.¹¹

The chemokine solution contained CXCL8 (Sigma, St. Louis, MO) in HBSS at a concentration of 1000 ng mL^{-1} (molecular weight = 8 kDa) and fluorescein isothiocyanate (FITC)-dextran (average molecular weight 10 kDa; Sigma, St. Louis, MO) at a concentration of 500 ng mL^{-1} (non-quenching concentration). The cell suspension contained human neutrophils at $2 \times 10^6 \text{ cells mL}^{-1}$ suspended in HBSS (Invitrogen, Carlsbad, CA) with 0.1% BSA (Sigma, St. Louis, MO). Neutrophils were allowed to sediment for 10 minutes. Phase-contrast and fluorescence microscopy images of the cell-containing chamber were sequentially acquired on an inverted microscope (Nikon ECLIPSE TE2000-U, Kanagawa, Japan) with a CCD camera (Hamamatsu ORCA-ER, Hamamatsu City, Japan), operated in its linear range (*i.e.* the gain set such that the brightest areas of the image were below saturation), at 2.5 minute intervals for two hours.

Cell tracking and analysis

In order to track individual cells, a program developed in-house in the programming platform MATLAB was used. This program allows the user to mouse-click on the position of a given cell in each phase-contrast image in the image sequence, displayed at high magnification on a computer screen, while the clicked positions are recorded to a file for further analysis. Other MATLAB routines developed in-house were used to calculate the vectors of cell position and cell speed in transformed coordinates (see text) and to analyze these values for correlation with estimated local CXCL8 concentration.

Results and discussion

Device design and operation

The microvalve chemotaxis device (μ VCD) consists of a stack of three elastomeric layers of which the middle layer is a thin PDMS membrane (Fig. 1a–e; see Experimental for details); we have recently reported the use of this design in a parallel mixer consisting of an array of microvalves.²⁵ The top layer of the μ VCD (termed the “fluidic layer”) contains the fluids and cells and the bottom layer (termed the “control layer”) contains dead-ended channels (termed “pneumatic lines”) that control the state of the microvalves (Fig. 1f). A microvalve (μ V) is opened or closed by applying negative pressure ($\sim 30 \text{ kPa}$) or positive pressure ($\sim 10 \text{ kPa}$) to its pneumatic line²⁵ (Fig. 1g). All features in the fluidic and control layers of the microvalve device were $\sim 110 \text{ }\mu\text{m}$ tall. The two microchambers, chamber A and chamber B, in the top layer were $600 \text{ }\mu\text{m}$ wide and 5 mm long. The membranes forming the microvalves had a circular ($500 \text{ }\mu\text{m}$ diam.) shape.

The principle of operation of our gradient generator is simple. First, dissimilar fluids are introduced in different chambers in the top layer of the device (Fig. 1f); next, the microvalve communicating the two chambers is opened, leading to diffusive mixing (*e.g.* forming a gradient) of the two fluids (Fig. 1g). A micrograph of the device used for these studies (chamber A filled with the food-coloring dye Allura Red) 20 min after the microvalve was open is shown in Fig. 1h to illustrate the formation of a radial gradient centered on the microvalve.

The sequence of steps needed to perform the chemotaxis assay is schematically depicted in Fig. 2. Arrows pointing up and down represent application of positive pressure ($P > 0$) or suction ($P < 0$), respectively, to the pneumatic lines. In preparation for the neutrophil chemotaxis assay, the set of four inlet/outlet microvalves (collectively termed μV_1) were opened while the microvalve separating microchambers A and B (termed μV_2) was kept closed (Fig. 2a). Next, chamber A (depicted dark grey in Fig. 2) was filled with CXCL8 solution (1000 ng mL^{-1} , molecular weight $M_r = 8 \text{ kDa}$) and FITC-dextran (500 ng mL^{-1} , $M_r \sim 10 \text{ kDa}$). Chamber B (light grey in Fig. 2) was filled with human neutrophils at 2×10^6

cells mL^{-1} . Neutrophils were allowed to sediment for 10 minutes, after which the four μV_1 were closed (Fig. 2b) in order to isolate chambers A and B from their inlets and outlets. Next (defined as time $t = 0$), μV_2 was opened, starting the formation of the gradient and causing the cells to migrate towards the microvalve (Fig. 2c). Phase-contrast and fluorescence microscopy images (to capture neutrophil movement and gradient formation, respectively) of chamber B were sequentially acquired at 2.5 minute intervals for two hours. The 10 kDa fluorescent dextran was used as an indicator for CXCL8 concentration and is assumed to diffuse at roughly the same rate as CXCL8 based on the similarity of their molecular weights.^{18,27}

As a principle, isolation of the device from the inlets is desirable for three reasons: (a) the gradient cannot be disrupted by hydrostatic pressure changes at the inlets (caused, for example, by evaporation or mechanical disturbances), so gradient formation is a purely diffusive process; (b) the total volume of the device is known with high precision, making gradient evolution amenable to computer simulation; and (c) the volume across which the gradient forms is very reproducible, thus reducing experiment-to-experiment variability. However, in the work presented here we did not evaluate the impact of these theoretical advantages on the actual chemotaxis assay outcome.

This microvalve design has two improvements over a design previously reported by Hosokawa and Maeda.²⁴ First, all inlets are on top of the device (the Hosokawa and Maeda microvalve device had inlets on top and bottom of the device); as a result, the bottom surface of our device is planar, allowing the microchambers to be easily visualized with an inverted microscope using an unmodified stage. Second, the top layer in this device is irreversibly (*i.e.* chemically) bonded to the pneumatic control system (in Hosokawa and Maeda's device, the layers were sealed by a reversible, conformal contact). Early experiments showed that the reversible seal that forms by conformal contact is weak and can be easily disrupted, which leads to leaks and unintended flow that disrupts gradient formation in chamber B. In addition to eliminating leaks, irreversibly bonding the fluidic layer to the pneumatic control system allows positive pressure to be applied to the microvalve, which ensures complete closure and faster operation of the microvalves.

Chemotactic behavior measurements

Cell migration was digitally tracked using a MATLAB script developed for this purpose. Additional MATLAB subroutines were developed to analyze the cell trajectories. Although there are a number of commercially-available software packages capable of tracking cell migration, it was deemed convenient to implement the cell tracking and the analysis on the same software platform. To analyze neutrophil migratory responses in the evolving gradient of CXCL8, we first translated the pixel coordinates of individual cells to rotated frames of reference (specific to each cell and image). Due to the radial symmetry of μV_2 , the center of μV_2 was designated as the origin (0,0) of the rotated coordinate system and is referred to as the "source". The measurement metrics are summarized and schematically depicted in Fig. 3. In the new reference frame, the axis that joins the origin with the position of cell n in image i is termed the "cell-source axis" (symbolized CA_i^n), which changes with time as the cell moves from one location to another; for instance, the cell-source axis for cell n at image 0, representing time $t = 0$, is termed CA_0^n (see Fig. 4c). The displacement vector of cell n in image i is defined as the difference between the coordinates of that cell in image i and those in the previous image $i - 1$; for any given cell n in image i , its radial velocity RV_i^n is defined as the component of the velocity vector parallel to the CA_i^n axis. The migration angle $\alpha_{\text{CA}_i^n}$ for cell n in image i is defined as the angle between the cell-source axis in the previous image CA_{i-1}^n and the displacement vector for that cell in image i . Thus, if cell n migrated

perfectly straight into the source in the time period elapsed between images i and $i - 1$, one would measure $\alpha_{CA}^n = 0$.

To test the μ VCD, 35 motile neutrophils were tracked for 120 minutes (a 49-image time-lapse sequence; the user need only click on the phase-contrast images). Shown in Fig. 4 are the 3rd ($t = 5$ min) and 13th ($t = 30$ min) pair of phase-contrast (Fig. 4a and c) and fluorescence (Fig. 4b and d) micrographs. To illustrate the typical trajectories, all the positions from $t = 0$ min to $t = 30$ min for a few cells (labeled with the roman numerals I through V) are superimposed as asterisks on the $t = 30$ min micrographs (Fig. 4c and d; the large circles, with the same roman numeral, denote the position of the cell in that frame). The initial cell-source axes for each cell, CA_0^I through CA_0^V , are depicted as arrows in Fig. 4c. The sequence clearly shows that the gradient develops with approximately radial symmetry around the source and that the cells display a biased migratory response toward the chemoattractant source. This bias is even more apparent when the trajectory of each cell is transformed to radial coordinates (taking the radial axis for *all* time points as the *initial* cell-source axis for each cell), as plotted in Fig. 4e. An overview of this data quickly reveals that (1) neutrophil migratory response is, on average, biased towards the source, (2) significant deviations from the initial CA_0^n axis (or any cell-source axis at different time points) occur often; and (3) there is considerable heterogeneity in the migration speeds and directions amongst the neutrophil population.

Extraction of estimated CXCL8 concentration from fluorescence intensity

Although the fluorescence intensity recorded is contributed by all the fluid on top of the cell culture surface, computer modeling shows that the fluorescence intensity at the cell culture surface follows a similar spatiotemporal evolution (data not shown), probably because the largest contribution to the total signal at any given fluid column comes from the surface layer (which is always closest to the microvalve than the fluid elements above). The relationship between dextran concentration and fluorescence intensity was assumed linear and calibrated using the two extreme values: the highest concentration of dextran (the known inlet concentration) and the absence of dextran correspond to the intensities seen in chambers A and B, respectively, right before opening the microvalve μV_2 . To reduce the influence of noise, the fluorescence intensity of any given pixel was assigned the average intensity in a surrounding 5×5 pixel grid. Next, we assumed that the 10 kDa fluorescent dextran and CXCL8 were diffusing at roughly the same rate (due to the similarity in their molecular weights); hence the normalized pixel intensity is equal to the normalized CXCL8 concentration. Moghe *et al.* used this assumption to calculate a diffusion coefficient (corrected for the M_r of CXCL8) of $2.54 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for CXCL8, based on the fluorescent profile of 10 kDa fluorescent dextran diffusing through a fibrin gel.²⁷ The value is in good agreement with the value of $2.09 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ obtained for the CXCL8 diffusion coefficient based on molecular weight considerations.²⁸

Unfortunately, the CXCL8 concentration at the cell's location cannot be extracted simply from the intensities of the pixels corresponding to the cell because the cells produce an artifactual peak in fluorescence. Hence, the fluorescence profile was fit with a fourth-order polynomial (that effectively "filters out" the cell's artifact) and the CXCL8 concentration was extracted from this fit. As an example, Fig. 4f shows the fluorescence intensities (jagged traces) and 4th-order fits (smooth traces) extracted from all time points ($t = 5$ –120 min) along the cell-source axis for cell IV at that time point, *i.e.* the curve corresponding to $t = 30$ min was obtained from the 13th image by reading the intensity trace along the CA_{13}^{IV} axis. Then the actual intensity value was obtained at the cell's position (shown as circle in Fig. 4f) from the 4th-order polynomial fit. We note that this neutrophil displays a clear directional bias towards the source (*i.e.* the microvalve), yet at one point (as the local CXCL8

concentration reaches a threshold value) the cell's migration becomes random – a behavior that was also observed in several other neutrophils using the same analysis.

One of the reviewers correctly identified this feature of our assay, and we agree it adds a degree of complexity in the analysis. First, even in devices that deliver static concentration gradients, the variability in cell responses is very large (and no two cells start at the same position nor, strictly speaking, concentration and gradient values). We have argued in this paper that *in vivo* all gradients are time-evolving by necessity (there is no sink), so this assay was designed to provide a system that is more realistic. The analysis tools we developed allow for tracking both the concentration and the position, so we are presently exploring whether we can infer interesting “time-dependent laws” for neutrophils migrating in spatio-temporal gradients by studying large numbers of neutrophils (to find redundant behaviors), which was beyond the scope of this article.

Correlations between cell migration parameters and local CXCL8 concentration

As opposed to other flow-based gradient generators^{18,19} that can deliver static concentration gradients, our μ VCD can only deliver time-decaying, point-source gradients. (In principle, slower decays could be achieved by intermittent opening/closure of the valve and other gradient shapes could be achieved using different valve geometries.) This feature is biologically attractive (*in vivo* gradients emanate from small numbers of secreting cells, which can be viewed as point sources) but inevitably complicates the analysis because no two cells are ever exposed to the same gradient. As gradient formation proceeds, the concentration at any given location in the device increases with time. Thus, in order to help the user find quantitative correlations between cell behavior and the local CXCL8 concentration, the software tool outputs various plots for any given cell n as a function of the local CXCL8 concentration (rather than time), such as plots (for cell n) of the speed V^n , radial velocity RV^n along the instantaneous cell-source axis, and the cosine of the migration angle ($\cos(\alpha_{CA}^n)$). Plots of V , RV , and $\cos(\alpha_{CA})$ for cells III, IV (the cell tracked in Fig. 4f) are shown in Fig. 5. Here, the velocities are averaged over five consecutive cell positions (*i.e.* cell positions from $i - 2$ and $i + 2$ were used to calculate the velocity assigned to position (i) to filter out small cell movements and user-introduced clicking errors. The cosine of the angle is more convenient than the angle itself as a parameter that conveys the turning behavior of the cell because, unlike the angle, the cosine does not depend on whether the cell turns to the left or to the right; a value of 1 indicates migration directly toward the source and a value of -1 indicating migration directly away from the source. This parameter is similar to what is known in the chemotaxis literature as the chemotactic index, which is widely used to characterize cell trajectories.²⁹

Finally, the software tool can also output histograms of V , RV , and α_{CA} for any chosen bracket of concentration values. For all the cells considered in the experiment of Fig. 4, the histograms of radial velocities (Fig. 6a) were clearly shifted to non-zero positive values (migration biased towards the source) for low CXCL8 concentrations but symmetrical around 0 (random migration) for high concentrations. This behavior is confirmed in the Rosetta histograms of the turning angle (Fig. 6c). Last but not least, histograms of the speed (Fig. 6b) reveal a considerable heterogeneity in migratory speeds, with average values around $2.5 \mu\text{m min}^{-1}$ at the lowest concentration bracket but shifting towards zero at the highest concentration brackets, suggesting that the neutrophils are switching from chemotaxis (directed migration) to chemokinesis (random migration). Further work to address the biological implications and origin of this striking difference in migratory behavior at different concentration brackets is underway.

Conclusions

The μ VCD is a powerful tool for studying cell migration in evolving gradients of chemoattractants. We have demonstrated that the chemotactic behavior of single neutrophils can be tracked over time in response to a radial, spatiotemporally-decaying gradient of CXCL8. A software tool was developed to automate the measurement of cell migration parameters (speed, radial velocity and turning angle) on dozens of cells and to extract correlations between the measured parameters and the concentration field. In our experiments, neutrophil migration displayed a large chemotactic component in CXCL8 gradients at local concentrations of up to 200 ng mL⁻¹, whereas chemokinesis played a more dominant role in neutrophil migration at higher local CXCL8 concentrations. In general, the combination of the μ VCD and the demonstrated analysis tools has several salient features important for *in vitro* cell motility assays. First, the μ VCD is amenable to rapid, inexpensive prototyping so that the design of the device can be easily modified. Second, a μ VCD experiment requires very few cells and small amounts of reagents or biological samples, which is critical when cells, reagents, or biological samples are in limited quantity or are expensive. Third, the μ VCD and analysis tools allow for obtaining single-cell migratory behavior data on large numbers of cells at high throughput, which can yield rich statistics on the measured parameters and their inherent variability across a large population of cells. Last but not least, the μ VCD can be applied to study the migratory behavior of other cell types.

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Fig. 1.

Fabrication and operation of the μ VCD. (a) Polydimethylsiloxane (PDMS) precursor is poured onto two masters containing photoresist microstructures (left and middle) and onto a spinning flat surface (right). (b) After curing, the PDMS replicas (labeled I and II) are peeled off the masters; a thin PDMS film forms onto the flat surface (right). (c) Replica II is bonded to the thin PDMS membrane. (d) Replica II is peeled off along with the PDMS thin film, which forms membrane-capped cavities in replica II. (e) Replica I and II are bonded together while applying suction to the cavities in replica II, which ensures that the membrane is not bonded to replica I after the bonding step. For device operation, the replica II cavities are used as pneumatic lines to apply negative pressure to the PDMS membrane, causing it to deflect. (f) Dissimilar fluids are loaded into the chambers while the valve is closed. (g) After opening the valves, a gradient starts forming. (h) Top-view micrograph of the microdevice 20 min after the microvalve has been opened, showing a gradient of Allura-Red dye (dark grey).

Fig. 2. Schematics (top-view and two different cross-sections) of the experimental procedure for a chemotaxis assay using the μ VCD. All four microvalves labeled μV_1 are operated through one pneumatic line (not shown), and a separate pneumatic line operates the microvalve μV_2 that communicates chambers A and B. (a) The four μV_1 are opened and the CXCL8 solution (dark grey) and the cells (light grey) are loaded into chambers A and B, respectively, through separate inlets while μV_2 is kept closed. (b) The four μV_1 are closed, such that chambers A and B are isolated from the outside and from each other. (c) The μV_2 is opened, which creates a fluidic communication between chambers A and B, starting the formation of a purely diffusive gradient (*i.e.* without flow) that induces chemotaxis of the cells up the gradient.

Fig. 3.

Definition of parameters used in characterizing directional migration in response to a point-like chemoattractant source (*i.e.*, the microvalve). The radial lines surrounding the source symbolize the development of the CXCL8 gradient. Cell-source axis (CA_i^n) – The axis that joins the origin (*i.e.*, source) with the position of cell n in image i . Migration angle ($\alpha_{CA_i^n}$) – The angle defined by CA_i^n (pointing to cell n 's position in image i) and CA_{i+1}^n (pointing to cell n 's new position in image $i + 1$). Speed or velocity (V_i^n) – The velocity vector of cell n in image i , averaged over five images (from image $i - 2$ to image $i + 2$). It is calculated by subtracting cell n 's position vector in image $i - 2$ from cell n 's position vector in image $i + 2$. Radial velocity (RV_i^n) – The component of V_i^n parallel to the CA_i^n axis.

Fig. 4.

Phase-contrast (a and c) and fluorescence (b and d) micrographs of a time-lapse sequence in a neutrophil migration experiment using the μVCD . Human neutrophils were introduced to microchamber B in HBSS with 0.1% BSA, at a density of 2×10^6 cells mL^{-1} and were allowed to settle for 10 minutes. A solution of $1 \mu\text{g mL}^{-1}$ CXCL8 (MW 8 kDa) and $0.5 \mu\text{g mL}^{-1}$ fluorescent dextran (average MW 10 kDa) in HBSS with 0.1% BSA was introduced into microchamber A (not seen, beyond the top of the images) prior to opening the microvalve μV_2 . The images correspond to $t = 5$ min (a and b) and $t = 30$ min (c and d). The location of the neutrophils (only five shown for clarity, labeled I through V) is indicated in all the images with shaded circles. The axes that join each cell with the source are termed “cell-source axes” (CA) and are different for each image i and for each cell n , CA_i^n . In (c), all the positions of cells I through V are indicated with coded asterisks and the CAs for the labeled cells at $t = 0$ (CA_0^{I} through CA_0^{V} , arrows) are superimposed. (e) Plot of the trajectories of neutrophils I through V over the course of 30 minutes; all the axes CA_0^{I} through CA_0^{V} have been rotated to a new frame of reference where the y axis (“radial axis”) coincides with each cell’s CA_0 and the source is at the origin. In the transformed

coordinates, chemotactic migration towards the source is clearly revealed as a trajectory directed towards the origin. (f) CXCL8 concentration values (jagged traces) along cell IV's cell-source axis CA_i^{IV} for all time points i corresponding to the experiment shown in a–d. The concentration values are calculated from the dextran conjugate's fluorescence intensity values (see Experimental); however, the concentration value at the cell position cannot be directly obtained from that point's fluorescence data because cells appear as artifactual fluorescence peaks. Instead, each concentration curve is fitted with a 4th-order polynomial (smooth traces), which effectively filters out the artifacts, and the concentration at the cell position is extracted from the 4th-order fits. For each time point, the cell position on the traces is indicated with a circle.

Fig. 5.

Heterogeneity in cell chemotactic behavior. The behavior of three cells from the experiment in Fig. 4 is depicted for cell III (first row), cell IV (second row), and cell V (third row). Shown are plots of speed averaged over five frames (V , first column), velocity along cell-source axis (RV , second column), and the cosine of the angle formed by the displacement vector (averaged over three frames) and the cell-source axis ($\cos(\alpha_{CA})$) (see Fig. 3 for definitions), as a function of CXCL8 concentration at the cell's position.

Fig. 6.

Histograms summarizing cell behavior are obtained directly from a stack of images (here, the same stack considered in Fig. 4). All occurring values of (a) radial velocity RV , (b) speed V and (c) migration angle α_{CA} (see Fig. 3 for definitions) were counted and separated into four groups according to one of four CXCL8 concentration ranges corresponding to that value. The values on the y axis are expressed as percentage of the total number of counts.