Supporting Information

A tuneable microfluidic system for long duration chemotaxis experiments in a 3D collagen matrix

Koceila Aizel,[a] Andrew Clark,[b] Anthony Simon,[b] Sara Geraldo,[b] Anette Funfak,[a] Pablo Vargas,[b] Jérôme Bibette,[a] Danijela Matic Vignjevic,[b] and Nicolas Bremond [a]

[a] *Laboratoire Colloïdes et Matériaux Divisés, CNRS, Chemistry Biology & Innovation, ESPCI Paris, PSL Research University, 10 rue Vauquelin, F-75005 Paris, France.*
[b] *Institut Curie, PSL Research University, CNRS, UMR 144, F-75005 Paris, France.*

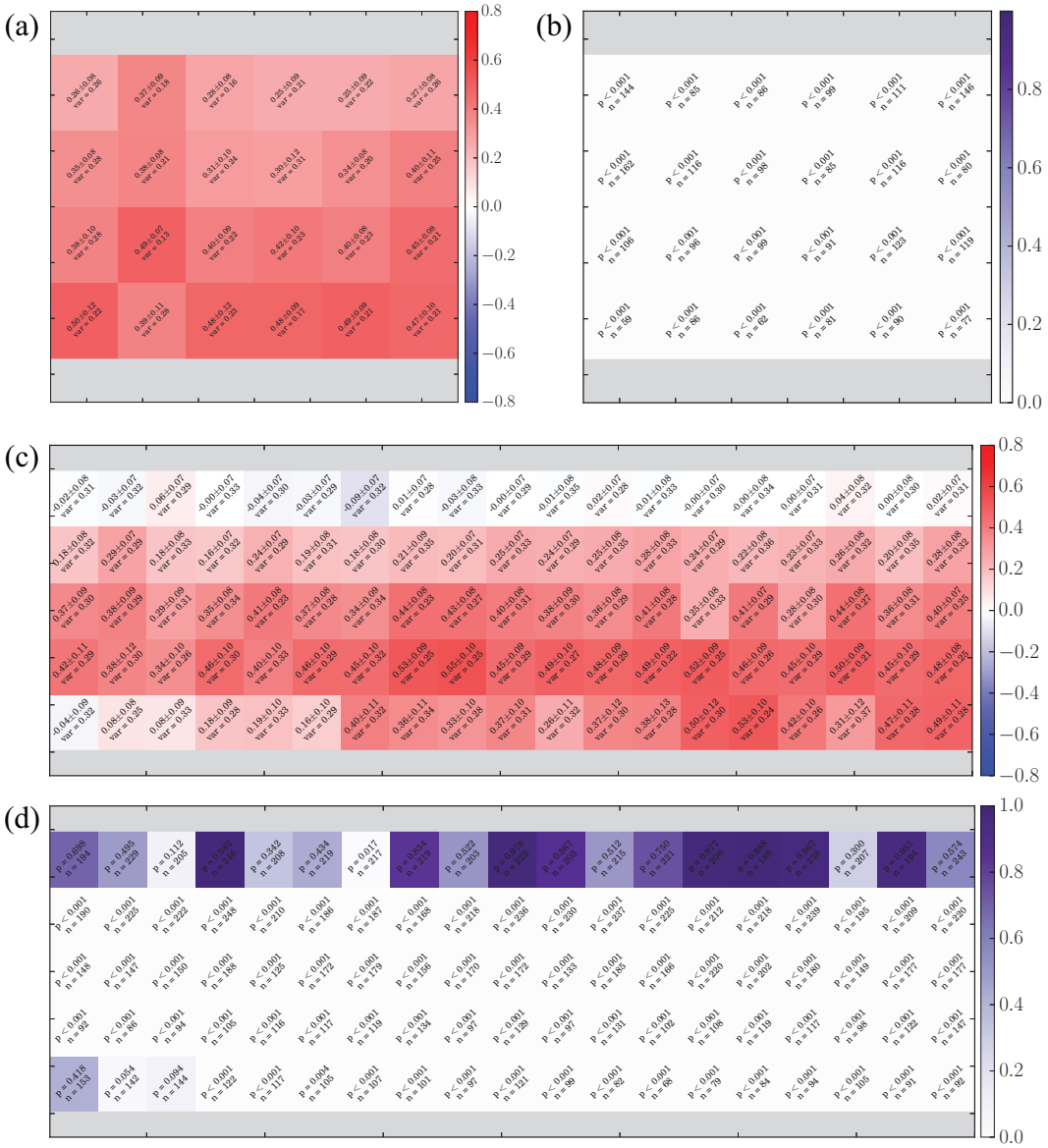


Figure S1: Migration trajectory statistics for primary mDCs in diffusion and convection-diffusion gradients of CCL19. (a) Binned heatmap of the chemotactic index (CI) of tracked cells from Fig. 4 (c), including mean ± 95% confidence intervals and variance (var) for each bin. (b) P-values from two-sided T-test for CI population of each bin in (a), with the null hypothesis that the mean $CI = 0$. The n value indicates the number of trajectories in each bin, across 4 independent experiments. (c) Binned heatmap of the chemotactic index (CI) of tracked cells from Fig. 5 (d), including mean ± 95% confidence intervals and variance (var) for each bin. (d) P-values from two-sided T-test for CI population of each bin in (c), with the null hypothesis that the mean $CI = 0$. The n value indicates the number of trajectories in each bin, across 7 independent experiments.

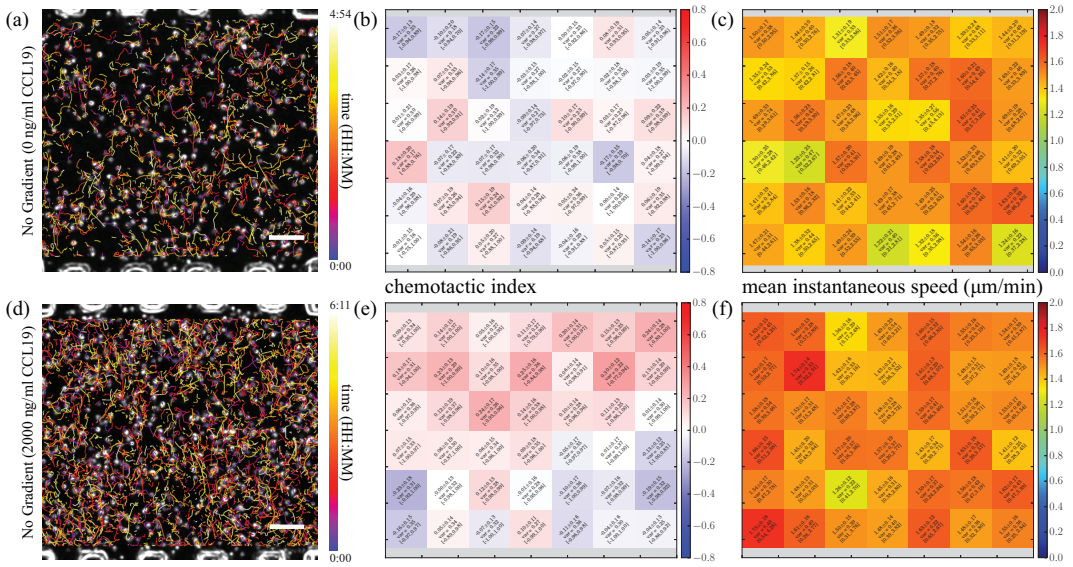


Figure S2: 3D migration of primary mDCs in diffusion-mode chambers in the absence of a chemokine gradient. (a,d) Tiled, pre-processed and tracked brightfield images of primary mDCs embedded in a 3D collagen matrix in a diffusion-mode chamber with medium with no CCL19 (a) or 200 ng/ml CCL19 (d) flowed above and below. (b,e) Binned heatmaps of the chemotactic index (CI) of tracked cells in (a,d), respectively, including mean ± 95% confidence intervals, variance (var) and the range ([min, max]) for each bin. Each binned square ($\sim$ 200 μm side length) represents the mean CI values for all tracked cells with starting positions that fall within the binned region. (c,f) Binned heatmaps of the mean instantaneous speed of tracked cells in (a,e), respectively, binned as in (b,e). The gray shaded area on the binned plots indicates the area between the edge of the channel (width = 1400 μm) and the edge of the region where trajectories were analyzed. Extreme edges were not included to avoid erroneous trajectories.

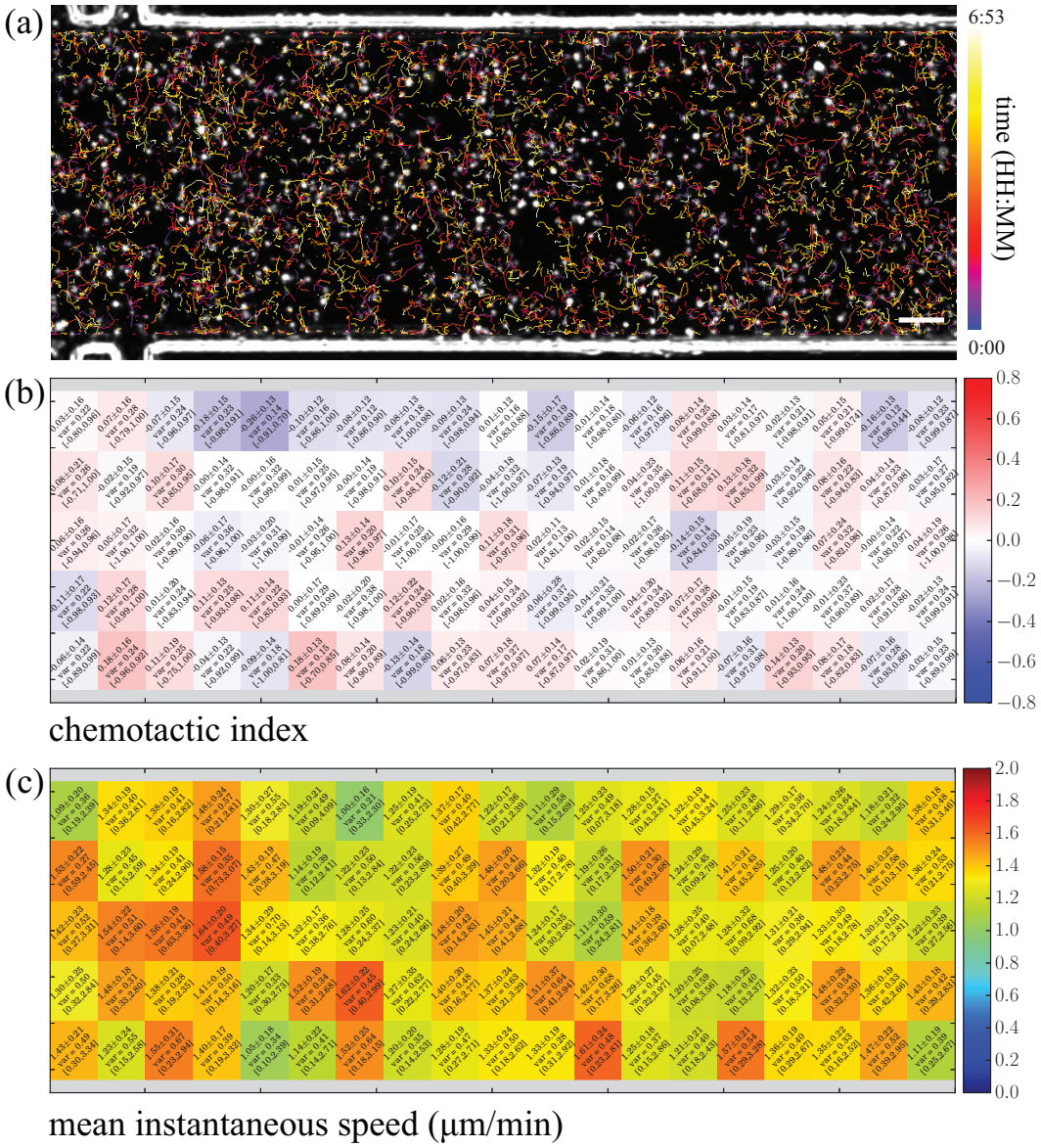


Figure S3: 3D migration of primary mDCs in the absence of a chemokine gradient with convective flow. (a). Tiled, pre-processed and tracked brightfield image of primary mDCs embedded in a 3D collagen matrix in a convection-diffusion mode chamber, with no chemokine gradient. Medium absent of chemokine was flowed through the chamber, resulting in no gradient, but fluid flow through the collagen network. Scale bar, 200 μm. (b). Binned heatmap of the chemotactic index (CI) of tracked cells in (a), including mean $\pm$ 95% confidence intervals, variance (var) and the range ([min, max]) for each bin. Each binned square ($\sim$ 200 μm side length) represents the mean CI for all tracked cells with starting positions that fall within the binned region. (c) Heatmap of the mean instantaneous speed of tracked cells in (a), binned as in (b). The gray shaded area on the binned plots indicates the area between the edge of the channel (width = 1400 μm) and the edge of the region where trajectories were analyzed. Extreme edges were not included to avoid erroneous trajectories.

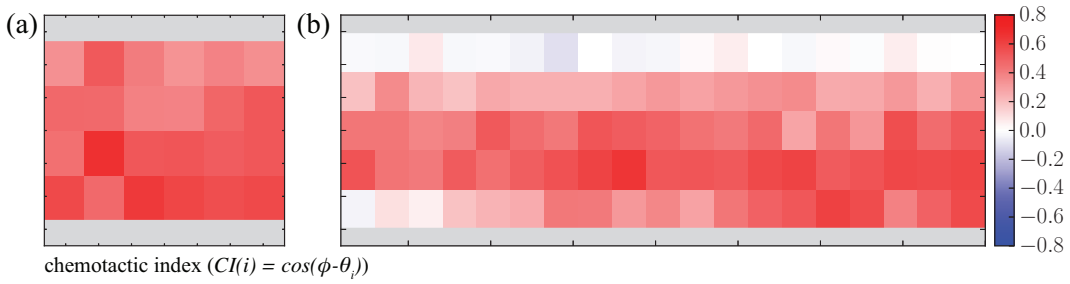
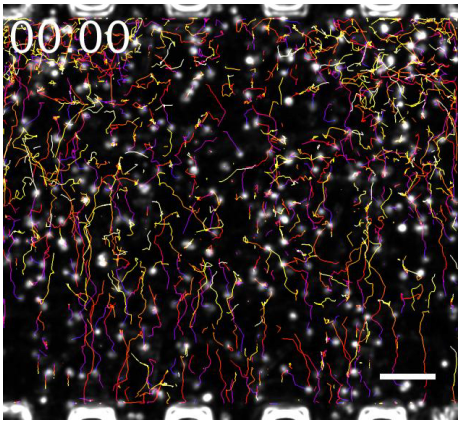
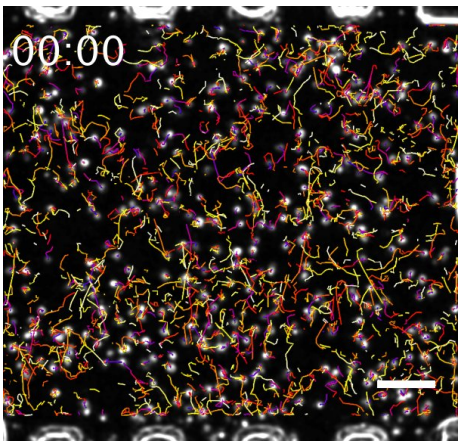


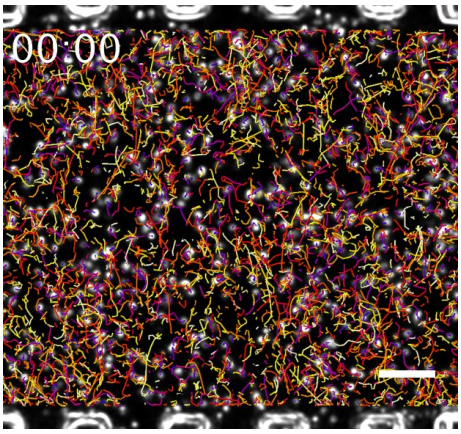
Figure S4: Chemotactic Index (CI) values using an alternate definition of CI. (a,b) Binned heatmaps of CI using the data shown in Figures 4(d) and 5(c) of the main text, respectively, where $CI(i) = \cos(\phi - \theta_i)$ and ϕ is the angle directly up the gradient (90°) and θ_i angle between start and end points of each track.



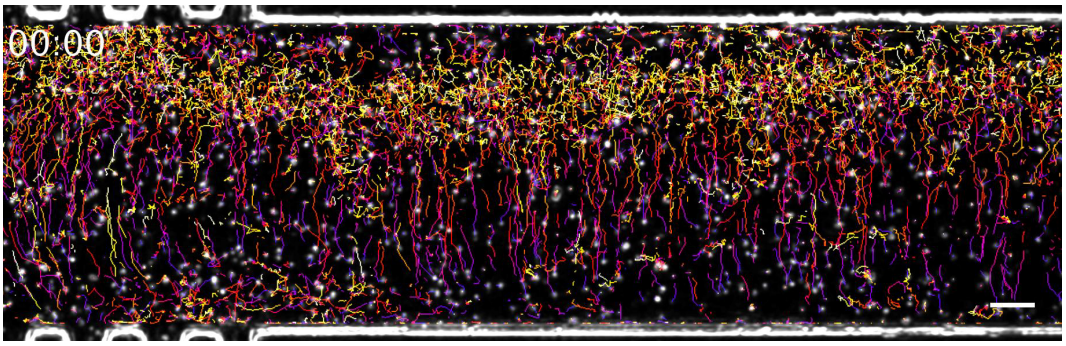
Movie S1: Timelapse image of mDCs migrating in a diffusion gradient of CCL19 (from Fig. 4(b)). Colored lines represent cells tracked from early (purple) to late (yellow) time points. HH:MM. Scale bar, 200 μm.



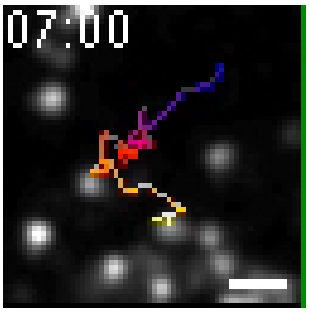
Movie S2: Timelapse image of mDCs migrating in a diffusion-mode chamber with no chemokine gradient (0 ng/ml CCL19; from Fig. S2(a)). Colored lines represent cells tracked from early (purple) to late (yellow) time points. HH:MM. Scale bar, 200 μm.



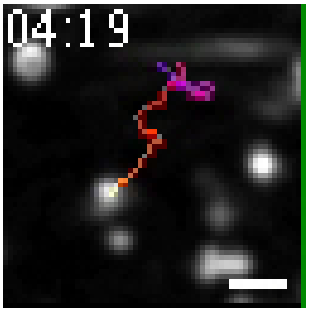
Movie S3: Timelapse image of mDCs migrating in a diffusion-mode chamber with no chemokine gradient (200 ng/ml CCL19; from Fig. S2(d)). Colored lines represent cells tracked from early (purple) to late (yellow) time points. HH:MM. Scale bar, 200 μm.



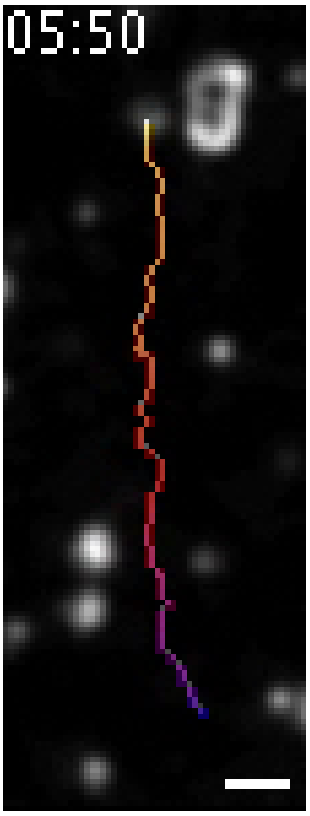
Movie S4: Timelapse image of mDCs migrating in a convection-diffusion gradient of CCL19 (from Fig. 5(c)). Colored lines represent cells tracked from early (purple) to late (yellow) time points. HH:MM. Scale bar, 200 μm.



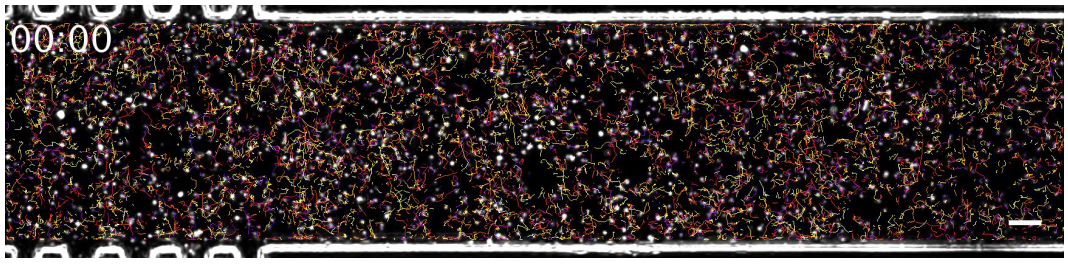
Movie S5: Timelapse image of a tracked mDC migrating in a convection-diffusion gradient chamber with a CCL19 gradient, in a region with no gradient and low chemokine concentration (from Fig. 5(c)-1). Colored lines represent cells tracked from early (purple) to late (yellow) time points. HH:MM. Scale bar, 20 μm.



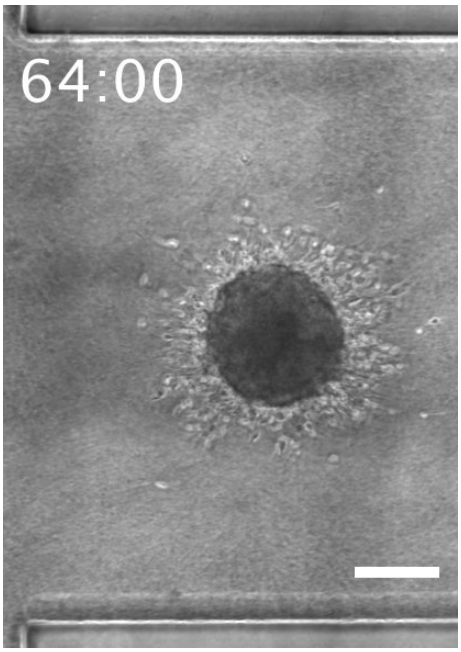
Movie S6: Timelapse image of a tracked mDC migrating in a convection-diffusion gradient chamber with a CCL19 gradient, in a region with no gradient and high chemokine concentration (from Fig. 5(c)-2). Colored lines represent cells tracked from early (purple) to late (yellow) time points. HH:MM. Scale bar, 20 μm.



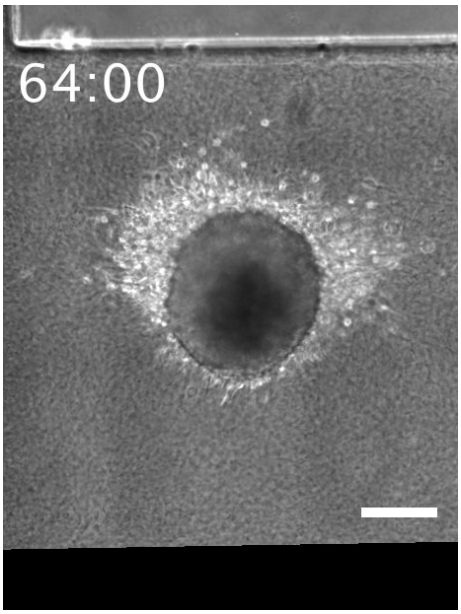
Movie S7: Timelapse image of a tracked mDC migrating in a convection-diffusion gradient chamber with a CCL19 gradient, in a region with a steep gradient (from Fig. 5(c)-3). Colored lines represent cells tracked from early (purple) to late (yellow) time points. HH:MM. Scale bar, 20 μm.



Movie S8: Timelapse image of mDCs migrating in a convection-diffusion gradient chamber with no chemokine gradient with convective flow (from Fig. S3(a)). Medium absent of chemokine was flowed through the chamber, resulting in no gradient, but fluid flow through the collagen network. Colored lines represent cells tracked from early (purple) to late (yellow) time points. HH:MM. Scale bar, 200 μm.



Movie S9: Timelapse image of a CT26 aggregate invading into collagen in the presence of 5% FBS without a concentration gradient (from Fig. 6(a)). HH:MM. Scale bar, 200 μm.



Movie S10: Timelapse image of a CT26 aggregate invading into collagen in the presence of a gradient of 0-5% FBS in convection-diffusion mode (from Fig. 6(d)). HH:MM. Scale bar, 200 μm.