Electronic Supplementary Material (ESI) for Integrative Biology

Retrotaxis of Human Neutrophils during Mechanical Confinement inside Microfluidic Channels

Bashar Hamza, Elisabeth Wong, Sachin Patel, Hansang Cho, Joseph Martel, and Daniel Irimia*
BioMEMS Resource Center, Massachusetts General Hospital, Harvard Medical School, and Shriners Hospital for Children, Charlestown, MA 02129.

Supplementary Information

Movie S1, ESI: Chemotaxis followed by retrotaxis in U-shaped channels. Neutrophil-like HL-60 cells with Hoechst-stained nuclei migrate along the arms of the U-shaped channels in a chemoattractant gradient (*fMLP*, 100nM). The gradient is visualized using a 3kD dextran-conjugated dye (shown in red): chemoattractant levels are highest at the tip of the U and diffuse outward towards the cell loading channel (not shown). After passing the tip of the U, which has the highest concentration of *f*MLP, neutrophils continue to migrate along the channel, in the opposite direction of the gradients.

Movie 2, ESI: Ph-Akt-GFP at the leading edge of the cell during retrotaxis. Differentiated neutrophil-like HL-60 cells transfected with a Ph-Akt-GFP reporter are used to determine cell polarity during retrotaxis. The GFP signal is visualized predominantly at the leading edge of the cell during both forward migration (not shown) and retrotaxis. Image slices were taken using a 100x/1.54 oil-immersion objective.

Movie 3, ESI: Retrotaxis of neutrophil-like HL-60 cell stops after encountering a zymosan particle. A differentiated neutrophil-like HL-60 cell transfected with a Ph-Akt-GFP reporter (green) loses cell polarity and stops retrotaxis after encountering and phagocytosing a zymosan particle (red). Channel width is 8 μm