# A Microfluidic Gradient Generating Device Integrated with Nanopatterned Matrices for Studying Guided Cell Migration

**Radu Reit**

**Biomedical Engineering, Georgia Institute of Technology**

*NNIN REU Site: NanoTech User Facility, University of Washington, Seattle, WA*
*NNIN REU Principal Investigator: Deok-Ho Kim, Bioengineering, University of Washington*
*NNIN REU Mentor: Nirveek Bhattacharjee, Bioengineering, University of Washington*
*Contact: rreit3@gatech.edu, deokho@uw.edu, nirveek@u.washington.edu*

## Abstract:

Migrating cells are inherently sensitive to a plethora of diffusible and immobilized cues, which are integrated to coordinate their directional migration in various contexts of organizational development, physiology and disease *in vivo*. However, the combined effects of these complex guidance cues on directional cell migration remains poorly understood, partly due to complete neglect of these combinational factors in most *in vitro* experimentation. Here, we developed a novel microfluidic platform with a nanostructured surface interface, allowing integrative stimulus delivery of chemoattractant factors and topographical cues to migrating cells. We combined nano-grooved substrates with open well microfluidic chambers to study the effects of chemoattractant factors and matrix topography on directional cell migration. Co-current and countercurrent flows of stromal derived factor-1 alpha (SDF-1$\alpha$) alongside bovine serum albumin (BSA)-labeled with fluorescent Rhodamine were used to study microfluidic gradient generation and the subsequent cell homing response. Using this platform, in a single experiment, we could simultaneously characterize cell migration and associated cell shape changes under well-defined variations in chemoattractant factors and matrix topography.

## Introduction:

Migrating cells are inherently sensitive to a plethora of diffusible and immobilized cues that are present in a combinatorial fashion, and are integrated by intracellular signal transduction machinery to coordinate their directional migration in various physiological and pathological contexts *in vivo*. A significant challenge faced by biomedical researchers is to study these factors combinatorially *in vitro* and understand their roles in determining cellular motility and directionality. Microfluidic chambers allow for a robust, high-throughput measurement technique for the study of chemotaxis and polarized cell movement [1]. Additionally, topographically amended substrates enhance sensitivity of migration, and allow integration of mechanical cues in experimental design [2]. However, the effect of combining these various extracellular cues and their subsequent effects on directed cellular homing remains vastly unexplored. Here we developed a novel microfluidic platform with a nanostructured surface interface, allowing integrative stimulus delivery of chemoattractant factors and topographical cues to migrating cells.

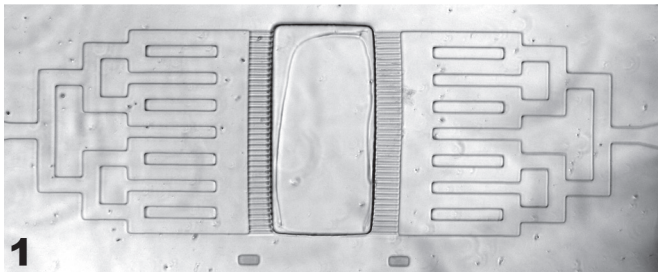
## Experimental Procedure:

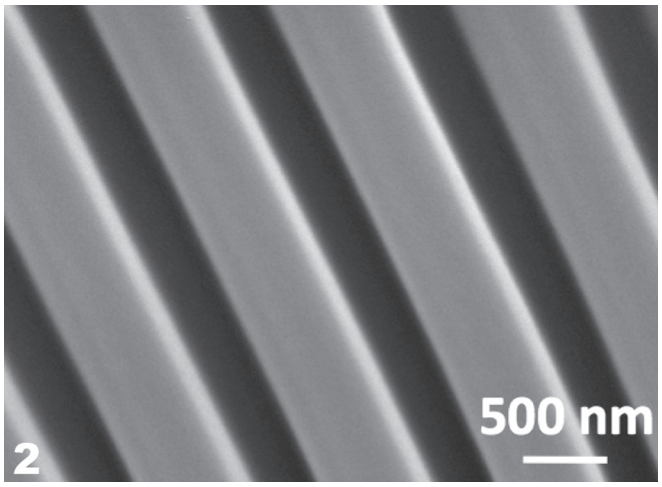
Nano-patterned polydimethylsiloxane (PDMS) was fabricated using soft lithography nanofabrication techniques to form patterns with 550 nm wide and 500 nm tall grooves, with a groove-to-ridge ratio of 1:1. Both flat and nano-grooved substrates were plasma-treated for 60 seconds at 650 mTorr and 60W, and then surface bound to a PDMS 4-well open-chamber microfluidic device. Co-current and countercurrent gradients were characterized using fluorescently labeled bovine serum albumin (BSA) flowed in at 1.6 $\mu$L/min.

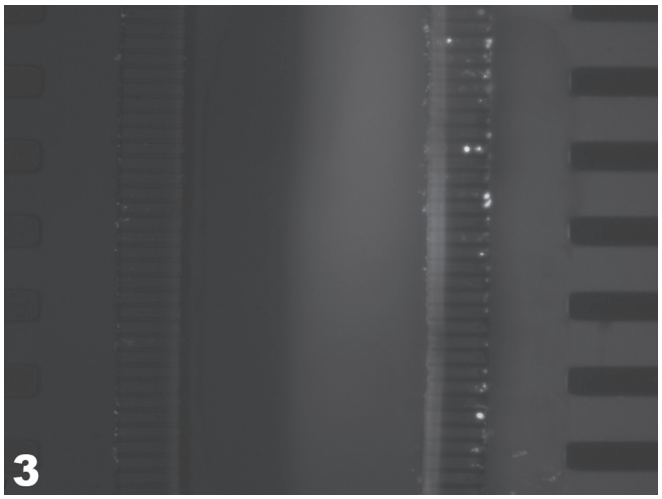
## Results and Conclusions:

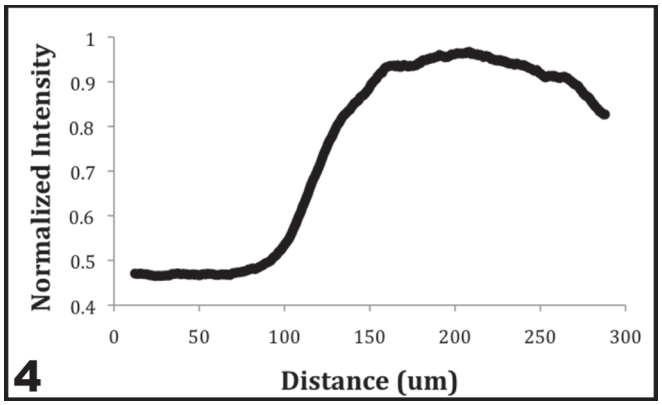
Microfluidic chambers and nanogrooved substrates were combined to create a device to test the effects of multiple extracellular factors synergistically *in vitro*. Figure 1 shows a well of the completed integrated device with the nanogrooved substrates along the bottom of the main cell-culture area shown in Figure 2. Microfluidic gradients were established, as shown in Figure 3, and later characterized for shape and linearity in Figure 4 at steady state.

In this study, we fabricated a high throughput microfluidic device using a nanopatterned PDMS substrate and an open-well microfluidic chamber. Protein flow gradients were characterized for linearity and used to optimize the flow rate for a biologically relevant chemotactic gradient. Overall, an easy-to-use, topographically mimetic microfluidic device was constructed for the combined study of chemotactic gradients and topography on directed cell migration.




## Future Work:

Upcoming experiments utilizing the microfluidic will focus on the role of cooperative and competing soluble factor gradients on the effects of directed cellular homing atop nanopatterned substrates. Co-current gradients will be established for the study of the synergistic effects of multiple soluble factors on cellular migration in the presence of multiple chemotactic gradients and compared with the observed individual soluble factor effects. Similarly, countercurrent gradients will be established for the study of the competing effect of chemotactic gradients and used to better understand the cellular sensitivity to specific chemoattractants. Both these experiments will serve to better understand the cell migration response to soluble factor gradients on a much more physiologically relevant substrate and will allow for the understanding of basic cellular migration in a more complex and relevant environment of microfluidic gradients.

## Acknowledgments:

The author would like to thank the National Science Foundation, and National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program, as well as the University of Washington Bioengineering Department and the University of Washington Nanotechnology User Facility (UW NTUF) for research support and funding.

## References:

[1] Keenan, T. M., Frevert, C. W., Wu, A., Wong, V., and Folch, A. (2010). A new method for studying gradient-induced neutrophil desensitization based on an open microfluidic chamber. Lab on a chip, 10(1), 116-22.

[2] Park, J., Kim, H.-N., Kim, D.-H., Levchenko, A., and Suh, K.-Y. (2012). Quantitative analysis of the combined effect of substrate rigidity and topographic guidance on cell morphology. IEEE transactions on nanobioscience, 11(1), 28-36.

*Figure 1: Phase 4x objective image of PDMS microfluidic chamber.*

*Figure 2: SEM image of 550 nm PDMS patterns within the exposed cell culture well.*

*Figure 3: Fluorescently labeled protein flown through the right hand channel.*

*Figure 4: Intensity profile of fluorescently labeled protein along the width of the microfluidic chamber.*