# Generation and applications of microfluidic gradients

Alex Groisman[1]

***Short Abstract* — Gradients of temperature and chemical concentration present in the environment and inside living organisms trigger directional migration and pattern formation and play an important role in the development and function of the organisms. Detailed studies of the gradient response require generation of well-defined gradients that can be maintained over extended intervals of time and modified, when needed. The gradient-making tools used in 20th century were rather inefficient and inaccurate, limiting the scope of experiments and quality of experimental data. With the advent of the modern microfluidic technology, it became possible to generate reproducible chemical gradients of various shapes and perform quantitative experiments on gradient response of various types of cells. We will discuss several microfluidic tools and techniques for making gradients of temperature and concentration of chemicals and gases dissolved in the medium and show how these tools are used to study chemotaxis, dose response, chemotropism, oxygen taxis, and thermotaxis.**

***Keywords* — gradients, microfluidics, chemotaxis, thermotaxis, oxygen taxis, dose response.**

20th century experimental tools used to generate gradients and study gradient responses were largely limited to diffusion chambers (such as Dunn and Zigmong chambers) and leaky capillaries. The gradients they generated were not completely reproducible and usually changed during the assays. Modern microfluidic technology enabled accurate control of flow and medium conditions in micro-chambers, making it possible to generate stable and reproducible gradients. A particular, a technique developed by the Whitesides group enabled generation of gradients with linear and polynomial shapes using pyramid-shaped gradient-making networks.[1]

This network was rewired by my group, making it more compact and capable of creating exponential gradients, which have several advantages over linear gradients.[2] Exponential gradients of an attractant fMLP were used to study chemotaxis of neutrophils-like cells, HL60.[3] This study analyzed the chemotactic trajectories of ~2000 individual cells and revealed the dependence of the chemotactic prowess of HL60 on the gradient and local concentration of fMLP. More recently, we applied exponential gradients of cAMP to study chemotactic response of social amoebae, *D. Discoideum*, revealing similar types of response to the gradient and local concentration of cAMP by this unicellular organism and establishing its ability to sense concentration differences as small as 1.25%.

An alternative approach to making gradients used by my group is by allowing molecular diffusion through a shallow channel between two continuously replenished channels with chemoattractant and plain medium. This device exposes cells to a stable gradient without exposing them to flow and is particularly advantageous for non-adherent cells such as yeast.[4] Application of gradients of pheromone to yeast cells in the device made it possible to observe and quantify their chemotropism and to perform a detailed study of their dose response.[4] We also used a version of the device with very shallow gradient chambers to study chemotaxis of *D. Discoideum* (a replacement of the popular under-agar assay) and to visualize nearly two-dimensional motion of cells during their chemotactic migration.

We used a technique of generation of gradients of temperature on a chip [5] to test the response of bacteria *E. coli* to variations of temperature. This thermotaxis assay had a throughput of $>10^4$ cells per hour and cells were found to congregate near 37 °C. We also applied the exponential gradient-making network to mix gases rather then liquids and to generate an exponential series of concentrations of oxygen and study the development of *E. coli* colonies at different oxygen tensions.[6] Finally, we used an off-chip gas mixer to generate gradients of oxygen concentration with a variety of functional shapes in a liquid medium.[7]

[1]Department of Physics, University of California, San Diego, La Jolla, CA 92093. E-mail: agroisman@ucsd.edu

[1] N. L. Jeon, *et al.*, "Generation of solution and surface gradients using microfluidic systems," *Langmuir* **16**, 8311-8316 (2000).
[2] K. Campbell, and A. Groisman, "Generation of complex concentration profiles in microchannels in a logarithmically small number of steps," *Lab Chip* **7**, 264-272 (2007).
[3] P. Herzmark, *et al.*, "Bound attractant at the leading vs. the trailing edge determines chemotactic prowess," *PNAS* **104**, 13349-13354 (2007).
[4] S. Paliwal, P. A. Iglesias, K. Campbell, Z. Hilioti, A. Groisman, and A. Levchenko, "MAPK-mediated bimodal gene expression and adaptive gradient sensing in yeast," *Nature* **446**, 46-51 (2007).
[5] V. Vandelinder, A. C. M. Ferreon, Y. Gambin, A. A. Deniz, and A. Groisman, "High-Resolution Temperature-Concentration Diagram of alpha-Synuclein Conformation Obtained from a Single Forster Resonance Energy Transfer Image in a Microfluidic Device," Analytical Chemistry **81**, 6929-6935 (2009).
[6] M. Polinkovsky, E. Gutierrez, A. Levchenko, and A. Groisman, "Fine temporal control of the medium gas content and acidity and on-chip generation of series of oxygen concentrations for cell cultures," *Lab Chip* **9**, 1073-1084 (2009).
[7] 7.M. Adler, M. Polinkovsky, E. Gutierrez, and A. Groisman, "Generation of oxygen gradients with arbitrary shapes in a microfluidic device," *Lab Chip* **10**, 388-391 (2010).