Supplementary Information

Numerical and experimental validation of single and dual gradient generation

We validated the single and dual gradient generation using an integrated experimental and numerical approach. Experimentally, we flowed fluorescence solution in the source channels, and blank buffer in the sink channels, and measured the fluorescence intensity in the microhabitat region using a fluorescence microscope. Numerically, we computed the steady state concentration field using the transport of diluted specie model in COMSOL Multiphysics, with boundary condition of constant concentration in source/sink channels and no flux at gel edge in a two-dimensional setting. The governing equations used were Fick's 1st and 2nd laws.

For single gradient simulation, we used device design in Figure 1A, FITC in one source channel, and buffer in the rest three side channels. FITC has a diffusion coefficient of $0.64 \times 10^{-4}\ m^2$/s. The initial values of FITC concentration, c1, was assumed 0 across the simulated field. Boundary conditions were: (i) right channel: c1 = 100 μM; (ii) the left channel: c1 = 0. The time dependent simulation was run for 120 minutes with a 1-minute step and the steady state concentration fields were achieved in about 70 minutes (as shown in Figure S1A). The steady state concentration gradient was linear across the two side channels (as shown in Figure S1B). We see slight discrepancy between the simulated and experimental data, which is likely caused by the 2D approximation in the simulation. While in experiment, the source /sink channels have finite depth, thus constant concentration assumption in a 2D setting is an approximation.

For dual gradient simulation, two chemical species (c1 and c2) were used. Species c1 represents FITC, while species c2 represents rhodamine b with a diffusion coefficient of $0.36 \times 10^{-4}\ m^2$/s. The initial values were c1 = c2 = 0 across the simulated field. Boundary conditions were: (i) top channel: c1 = 100 μM, c2 = 0; (ii) right channel: c1 = 0, c2 = 100 μM; (iii) bottom and left channels: c1 = c2 = 0. (See Figure 1A) The time dependent simulation was run for 90 minutes with a 1-minute step, and the steady state concentration fields (Figure 1B) were achieved in about 70 minutes as shown in Figure S2.

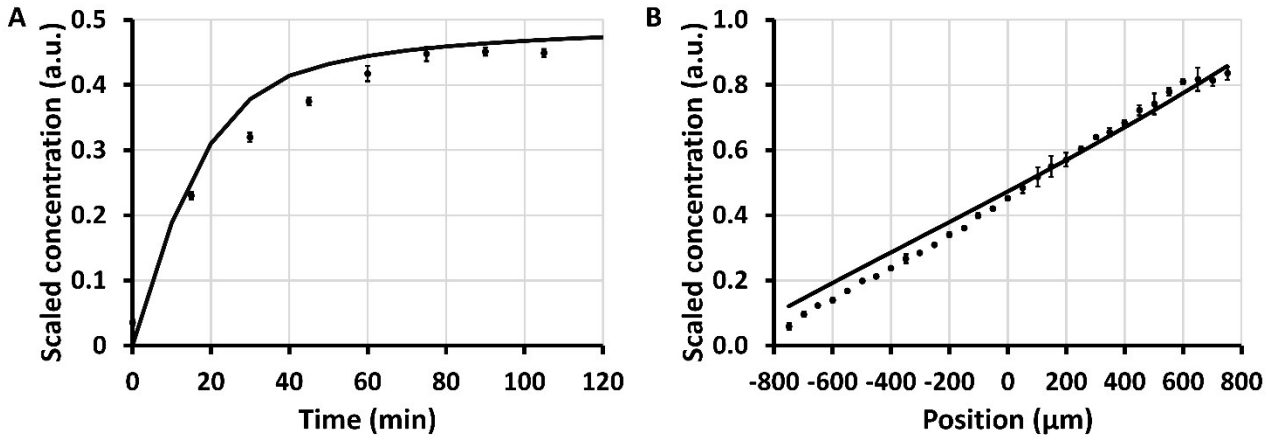

Figure S1. Experimental versus simulation results of single gradient generation A. FITC concentration from experiments (dots) and numerical simulation (solid line) at the middle of the microhabitat array over time. Y axis is the concentration scaled between source channel as 1 and sink channel as 0 and x-axis is time. B. FITC concentration from experiments (dots) and numerical simulation (solid line) in the microhabitat region across channels at t = 120 min, which describes the steady state gradient. Y axis is the concentration scaled between source channel as 1 and sink channel as 0 and x-axis is position across channels with the middle of the microhabitat array as 0 µm.

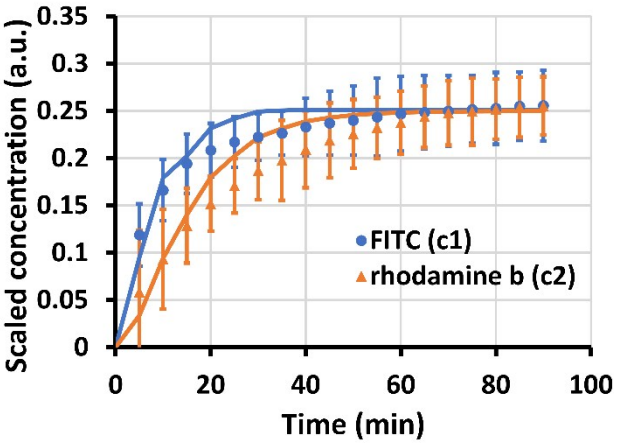

Figure S2. Experimental versus numerical results of dual gradient generation FITC and rhodamine b concentration at the middle of the microhabitat array over time. Y axis is the concentration scaled between source channel as 1 and sink channel as 0. Dots and lines represent experimental results and numerical results respectively.

Growth of *C. reinhardtii* under single nutrient gradient

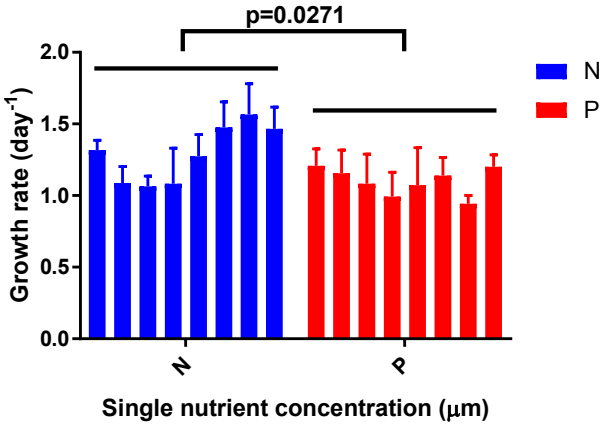

Figure S3. *C. reinhardtii* growth rates under single nutrient gradient (rearrangement of Figure 4A) Growth rates under single N gradient are shown in blue bars, under single P gradient are shown in red bars. For each nutrient, a single bar represents a nutrient concentration as described in Figure 4A, namely, 7.5, 12.5, 17.5,22.5, 27.5, 32.5, 37.5 and 42.5 μM from left to right. Error bars represent standard error of the mean (SEM). Difference was observed between overall growth rates under the two nutrients (p value = 0.0271 in unpaired t-test).

Movie S1. *C. reinhardtii* cells swimming in a microhabitat. This is a 20 frames per second bright field movie taken using 40x objective showing that *C. reinhardtii* cells can swim freely within the 100 μm × 100 μm × 100 μm microhabitat.

Movie S2. *C. reinhardtii* cells growth and multiple fission in a microhabitat. Time laps images were taken using 10x objective. The first frame was at t = 0 hour, the second frame was at t = 17 hours. Starting frame 3, there were five consecutive frames taken starting at 19.75 hours with 1 hour interval. Starting frame 7, images were taken with 30 min interval until the end of the movie.