Universal Microfluidic Gradient Generator

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Essential for our understanding of cellular responses to biochemical heterogeneities in their microenvironment is our ability to consistently replicate and systematically alter chemical concentrations around cells *in vitro*. While microfluidic devices have shown unmatched capability in generating linear stable chemical gradients, no systematic method exists today for replicating more complex gradients. Here we demonstrate a *universal* approach for generating stable gradients of any profile, starting from only two input concentrations, by the use of mathematical theory and microfluidic principles.

Precise complex gradients are directly relevant for bacteria and eukaryotic cell adaptation and migration. Spatial chemical gradients are also important for immune cells migrating against intruders[1,2], axons regenerating towards their connection target[3], and embryonic cells differentiating in response to morphogens[4].

We restrict the diffusion between two parallel streams of distinct concentrations flowing at low Reynolds number (laminar flow) by using a series of dividers in the longitudinal direction of the channel. At steady state, the target concentration profile is generated at the outlet, in direction transversal to the channel, by the parallel flow of several sub-streams (Figure 1). The underlying working principle is the complete mixing of controlled fractions of adjacent streams. A new concentration $C_{n,m}$ ($m=\overline{1,n}$) is generated by the mixing of precise fractions from streams of concentrations $C_{n-1,m-1}$ and $C_{n-1,m}$. In order for such mixing to occur regularly at any positions across one level, a set of constrains for the position of dividers at two consecutive levels has to be considered:

$$0 < u_{n,0} < u_{n-1,0} < u_{n,1} < u_{n-1,1} < \ldots < u_{n,m-1} < u_{n-1,m-1} < u_{n,m} < u_{n-1,m} < \ldots < u_{n-1,n-1} < u_{n,n} \qquad \text{(Eq.1)}$$

Using mass conservation equations:

$$C_{n,m} \cdot (u_{n,m} - u_{n,m-1}) = C_{n-1,m-1} \cdot (u_{n-1,m-1} - u_{n,m-}1) + C_{n-1,m} \cdot (u_{n,m} - u_{n-1,m-1}) \qquad m=\overline{1,n} \qquad \text{(Eq.2)}$$

we demonstrate that for any given set of $C_{n,i}$ and $u_{n,i}$, there exists at least one set of $C_{n-1,i}$ and $u_{n-1,i}$ ($i=\overline{0,n}$) that verifies the compatibility condition from Equation 1 (see supplementary information). By using mathematical induction principles, this is also proof that for any array of $C_{N,i}$ ($i=\overline{0,N+1}$) concentrations at the output, a complete set of concentrations and divider

positions can be found for generating these concentrations, starting from two distinct input concentrations.

One interesting remark emerging from the mathematical demonstration is that an infinite number of design solutions satisfying the divider position constrains and mixing equations are possible. These solutions can be systematically represented by the introduction of a set of coefficients α ($0 < \alpha_{ij} < 1$) at our disposal (see supplementary information).

Based on these principles we developed a computer algorithm and built microchannels with dividers for generating four gradients, the equivalent of *power, exponential, error,* and *cubic root* functions (see supplementary information). Comparisons between the experimental and predicted concentration profiles showed differences less than 0.05 between the normalized experimental and theoretical concentrations for the microfluidic devices in which the output is reconstituted from N=10 unequal sub-streams (Figure 1b-e). The predicted error of gradient reproduction was calculated for the particular choice of divider position to be less than 0.1.

In conclusion, a systematic approach for designer gradients at microscale is presented. A mathematical apparatus was developed consisting of two parts, one for demonstrating the universal applicability of our approach and one for extracting the design details starting from any chosen output.

1. Wilkinson, P. C. Assays of leukocyte locomotion and chemotaxis. *Journal of Immunological Methods* **216**, 139-153 (1998).
2. Jeon, N. L. et al. Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. *Nature Biotechnology* **20**, 826-830 (2002).
3. Goodhill, G. J. Mathematical guidance for axons. *Trends in Neurosciences* **21**, 226-231 (1998).
4. Gurdon, J. B. & Bourillot, P. Y. Morphogen gradient interpretation. *Nature* **413**, 797-803 (2001).

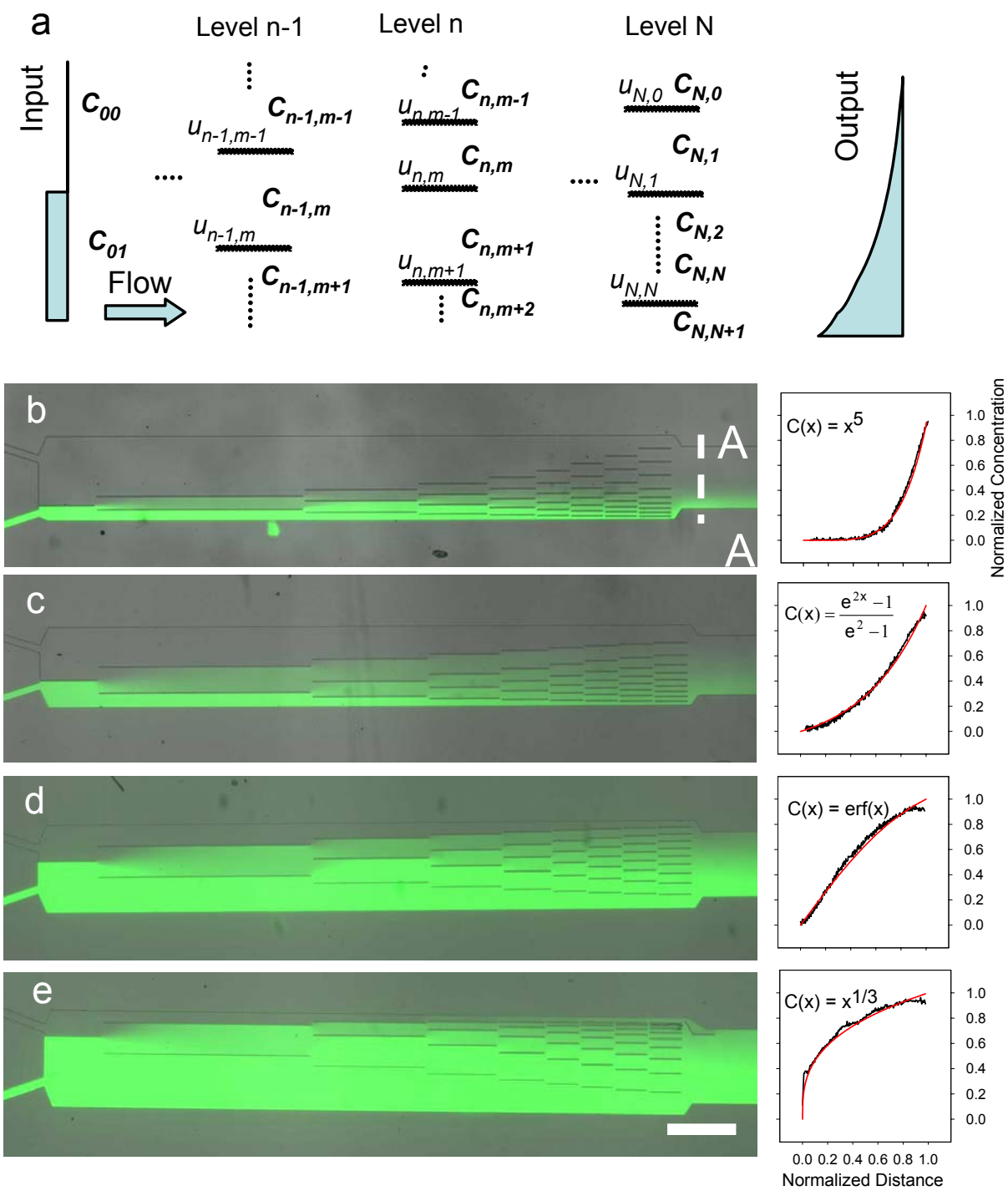
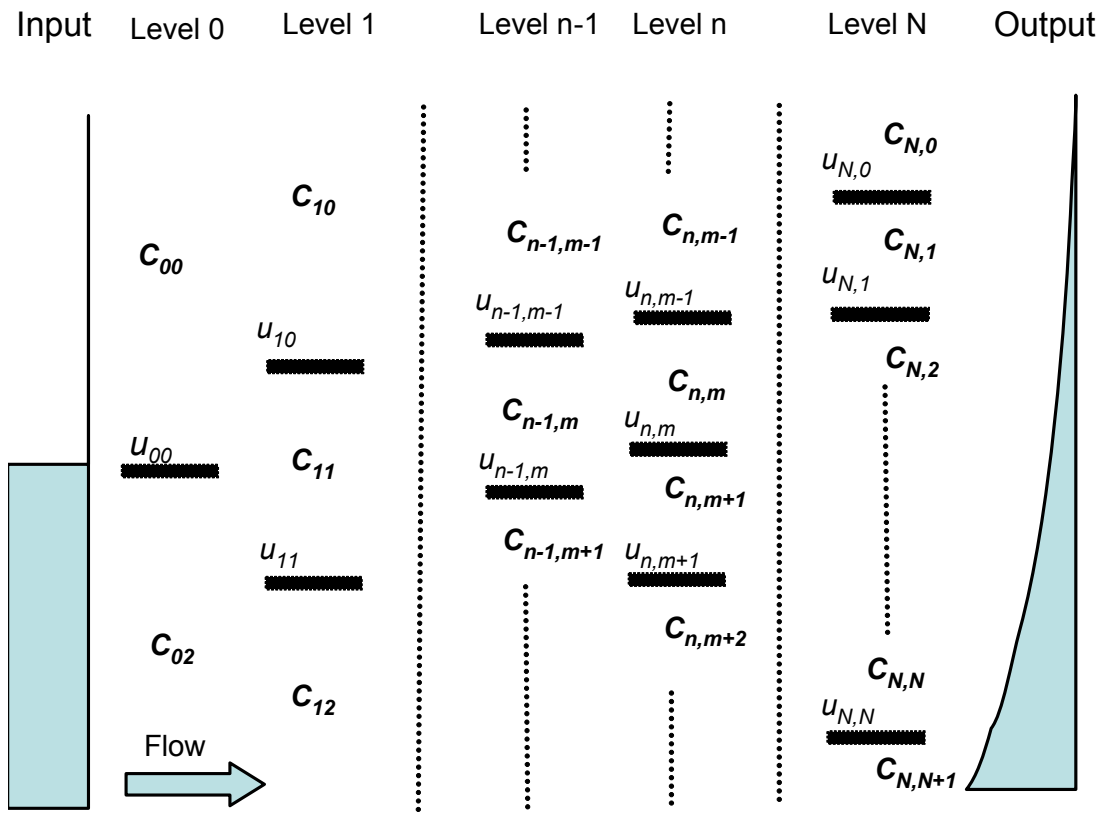


Figure 1. Universal gradient generator. a, The sub-stream concentrations and position of the dividers at an intermediate level inside the microchannel. b-e, The resulting microfluidic structures, images of steady state fluorescent gradients inside the devices, and comparisons between experimental and theoretical normalized concentration profiles for fifth power (b), exponential (c), error (d), and cubic root functions (e). Fluorescence intensity was measured at level AA for all channels. Scale bar is 500μm.

Supplementary information



Supplementary figure 1. Schematics of the dividers and concentrations in the microchannel for generating a concentration gradient at the output.

Propositon 1. Considering a set of concentrations $C_{n,i}$ $(i=\overline{0,n+1})$ and dividers positions $u_{n,i}$ $(i=\overline{0,n})$ known (supplementary figure 1), with $0 \le C_{n,0} < C_{n,1} \ldots < C_{n,n+1}$ *and* $0 < U_{n,0} < U_{n,1} \ldots < U_{n,n}$, the mass conservation equations

$$C_{n,m} \cdot (u_{n,m} - u_{n,m-1}) = C_{n-1,m-1} \cdot (u_{n-1,m-1} - u_{n,m-1}) + C_{n-1,m} \cdot (u_{n,m} - u_{n-1,m-1}) \qquad m=\overline{1,n} \qquad \text{Eq.S1}$$

have a unique solution $u_{n-1,i}$ $(i=\overline{0,n-1})$ that verifies the condition

$$0 < u_{n,0} < u_{n-1,0} < u_{n,1} < u_{n-1,1} < \ldots < u_{n,m-1} < u_{n-1,m-1} < u_{n,m} < u_{n-1,m} < \ldots < u_{n-1,n-1} < u_{n,n} \qquad \text{Eq.S2}$$

if and only if the following inequality holds:

$$0 \le C_{n,0} \le C_{n-1,0} \le C_{n,1} \le C_{n-1,1} \ldots \le C_{n-1,n} \le C_{n,n+1}. \qquad \text{Eq.S3}$$

Proof: We start with the first equation that can be written in the form

$$u_{n-1,m-1} = \frac{C_{n,m} \cdot (u_{n,m} - u_{n,m-1}) + C_{n-1,m} \cdot u_{n,m} - C_{n-1,m-1} \cdot u_{n,m-1}}{C_{n-1,m-1} - C_{n-1,m}} \qquad \text{Eq.S4}$$

By plugging $u_{n-1,m-1}$ into the compatibility condition $u_{n,m-1} < u_{n-1,m-1} < u_{n,m}$ we obtain after a couple of reductions

$$C_{n-1,m-1} \cdot (u_{n,m} - u_{n,m-1}) \le C_{n,m} \cdot (u_{n,m} - u_{n,m-1}) \le C_{n-1,m} \cdot (u_{n,m} - u_{n,m-1}) \qquad \text{Eq.S5}$$

which implies, considering $u_{n,m-1} < u_{n,m}$ that

$$C_{n-1,m-1} \le C_{n,m} \le C_{n-1,m} \,. \qquad \text{Eq.S6}$$

It can be seen immediately that the argument is reversible, therefore we obtain the equivalence stated in proposition 1. The restrictions we apply on the order of these concentrations do not affect the universality of the approach, because any continuous function can be decomposed into simpler functions on intervals. Several streams of different concentration profiles can then be combined to reproduce a more complex function[1].

Proposition 2. One way to write the complementary equations to the mass conservation equations is the following form:

$$C_{n-1,m} = \alpha_{n-1,m} \cdot C_{n,m} + (1 - \alpha_{n-1,m}) \cdot C_{n,m+1} \qquad \text{Eq.S7}$$

where $\alpha_{n,m}$ ($0 < \alpha_{nm} < 1$) would be coefficients at our disposal.

Proof: This equation contains implicitly the condition from Equation S6 and assures us that we can solve the system at each level obtaining positions for dividers which verify the compatibility condition. The restrictions for particular *α* values are dictated mainly by practical considerations, like the minimal distance between dividers that can still be resolved by the microfabrication techniques.

Proposition 3. The minimum number of sub-streams with concentrations $C_{N,,i}$ which would reconstitute the target concentration profile with necessary precision ε can also be calculated

Proof : Due to the fact that the domain of the function f is the compact interval $[C_{00},C_{01}]$, f is more than continuous, namely uniform continuous. Thus, for any ε we could find a particular *N* such that for any x_1,x_2 in the interval $[u_{N,0},u_{N,N}]$, with $|x_1 - x_2| < \frac{u_{N,N} - u_{N,0}}{N}$, then $|f(x_1) - f(x_2)| < \varepsilon$. In other words, for a particular desired precision ε of the approximation of a concentration profile, we can determine the minimum number of microfluidic streams necessary to reconstitute that concentration profile. In practice narrower streams are used in the regions of higher gradient steepness. The experimental results usually show better precision than predictions as a result of gradient smoothing by diffusion after the last set of dividers.

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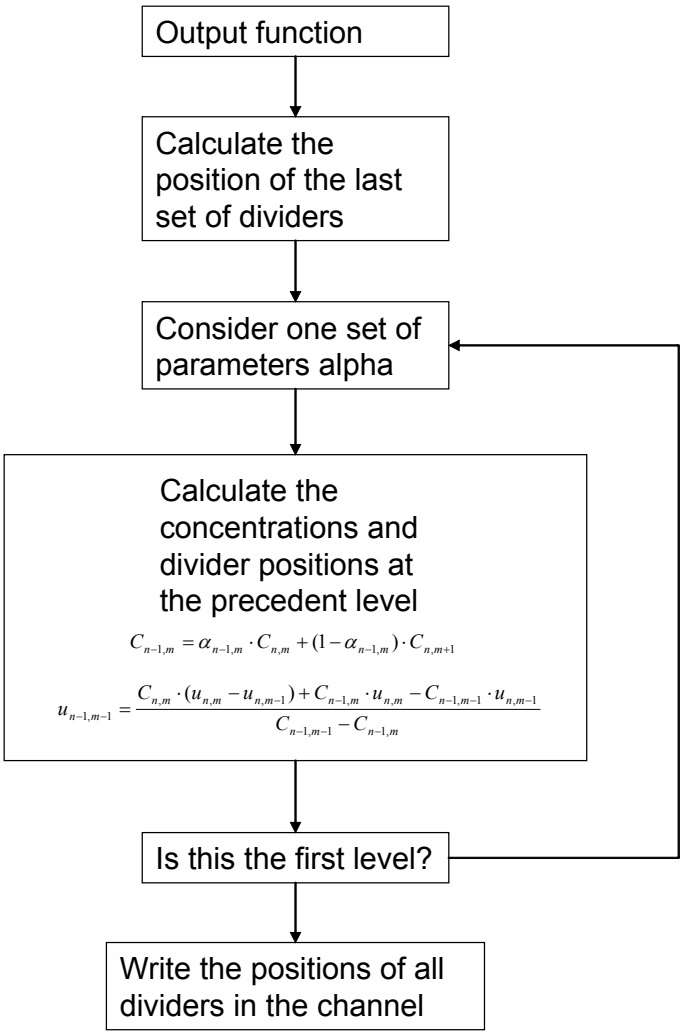
[1] Dertinger, S. K. W., Chiu, D. T., Jeon, N. L. & Whitesides, G. M. Generation of gradients having complex shapes using microfluidic networks. *Analytical Chemistry* **73**, 1240-1246 (2001).

Schematic protocol for calculation of the position of the dividers inside the main channel.



Supplementary figure 2. Logic diagram for calculating the position of the dividers in the channel for a desired output concentration gradient.

The length of the dividers in the longitudinal direction parallel to the flow of the dividers was calculated to match the velocity of the streams and the time required for 99% complete mixing by diffusion of the merged streams at each level.

Experimental method. Standard microfabrication technology has been used to fabricate 10μm wide dividers in 400μm wide and 20 μm high channels in polydimethylsiloxane (PDMS) on glass. Briefly, a 20μm layer of SU8 (Microchem, Newton, MA) was spun on a silicon wafer and photopatterned according to manufacturer's instructions. PDMS (Sylgard 184; Dow Corning, Midland, MI) was prepared according to the manufacturer's instructions and cast over the photoresist mold to create complementary microchannels in PDMS. Through holes, defining the inlets and outlets, were punched using a beveled 25-gauge needle. The bonding surfaces of the PDMS and a regular glass slide (1×3 inches – Fisher Scientific, Pittsburgh, PA) were treated with oxygen plasma (150 mTorr, 50 W, 20 s) produced in the parallel plate plasma asher (March Inc., Concord, CA). A good seal between the PDMS and glass was achieved by heating the assembly at 75ºC for 10 minutes on a hotplate.

Concentration profiles have been tested in four microfluidic devices using Fluorescein isothiocynate (FITC) dyes and distilled water as starting solutions. Gravitational flow was employed to maintain a slow (10 μm/s), controlled fluid velocity inside the microchannel. The fluorescence intensity was quantified in micrographs at 100 μm downstream from the last set of dividers (section AA) and equivalent concentration calculated based on a linear dependence between concentrations and fluorescence intensities.

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