Modular microfluidics for gradient generation

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This paper describes a modular approach to constructing microfluidic systems for the generation of gradients of arbitrary profiles. Unlike most current microfluidic-based systems that have integrated architectures, we design several basic component modules such as distributors, combiners, resistors and collectors and connect them into networks that produce gradients of any profile at will. Using the system as a platform we can generate arbitrary gradient profiles that are tunable in real time. The key advantage of this system is that its operation is based on prefabricated components that are relatively simple. Particularly for non-specialists, the modular microfluidic system is easier to implement and more versatile compared to single, integrated gradient generators. The disadvantages associated with this system is that the total amount of liquids used is rather large compared with single chip-based systems. The system would be useful in simulating environments in vivo, e.g., studying how cells respond to temporal and spatial stimuli.

Introduction

Gradients of biomolecules play important roles in biological processes such as chemotaxis, morphogenesis, and axon pathfinding. To study these phenomena in vitro, many types of gradient generators have been developed. Some devices make use of molecules diffusing between two neighboring channels, or by introducing a flush of solutions of increasing volumes. These systems usually make nonlinear gradient profiles. Whitesides et al. demonstrated a microfluidic network that can achieve complex gradients through repeatedly splitting and mixing of two starting solutions. Other devices involve serial dilution, bringing different volumetric ratios of pre-mixed solutions together, or adding parallel dividers to split solutions in different positions to generate monotonic arbitrary gradient profiles. Apart from these, Wu et al. proposed a new method that allows generation of static arbitrary gradients by designing the path of channels to pick up desired concentrations from a preformed gradient.

Although our abilities in generating gradients have greatly improved with the use of microfluidic channels, existing methods for making gradients still have several drawbacks. In biology, the profiles of some gradients may turn out to be too complicated to describe in simple functions in space and time, due to complex physiological processes. For example, during the development of the mammalian pituitary gland, adjacent tissues around the gland establish multiple gradients to induce primordial pituitary cells to differentiate into different cell types. Reproduction of such gradients in the laboratory requires both spatial and temporal control over gradients. According to most current microfluidic gradient generators, the more complex the profile is, however, the more complex the architecture or design of these devices will become. Gradient generators that have two inlets for pre-diluted solutions cannot generate a gradient with a concave or a convex profile. Another drawback of gradient generators based on single integrated chips is that each design is intended for a particular use. Once the microfluidic chips are fabricated, most of them can only generate one type or a limited number of types of profile. (For example, once a master of a gradient generator is fabricated, it allows the generation of gradient of a set size.) A different size of gradient would require a completely different master and therefore new fabrication in a clean-room facility. Although some can operate dynamically, the resulting types of gradient are limited. Because of this limitation, any modification, imperfection or injury of existing chips will require the re-fabrication and/or redesign of the entire system, raising the difficulty and costs (both in time and money) of experiments.

Here, we use a modular approach in constructing a gradient generator to overcome some of the drawbacks in integrated microfluidic systems. In this approach, modules are units that have standardized dimensions; the modules can be assembled easily using macroscopic tubing. There have been applications of modular approaches in some systems where functional modules or units carry out single assays (like sample concentration or electrophoresis); our approach is different from these systems in that we attempt to decompose and distribute the functions of complex fluidic networks into individual, standardized parts that carry singular, well-defined functions. We apply the modular approach in gradient generation and build the system with a set of individual chips. The chips contain microfluidic channels with simple patterns. We use multiple chips connected via plastic tubes, instead of single, integrated chips containing masters of microfluidic networks. In this approach, basic chips or modules in connection can be added, subtracted, or replaced manually, making the construction for complex functions straightforward. The modules can be fabricated rapidly and in large numbers, and the whole system can be assembled and disassembled easily.

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Hence, the modular system is more sustainable compared to the integrated system, which can be destroyed by local damage. In our system, once the modules are fabricated, there is no need for the use of the clean room at all. Our system still retains some of the advantages associated with integrated chips. Both are portable, and provide a high surface-to-volume ratio environment for fluids in the functional areas. The system, however, has some disadvantages at the same time, such as the large overall fluid consumption as a result of macroscopic tubes that connect the modules.

The modular system we construct can generate complex gradient profiles of essentially any types; these profiles can also be dynamically modified into other profiles (e.g. from concave to convex types). To the best of our knowledge, this type of dynamic/all-purpose gradient from two starting solutions has not been demonstrated experimentally, although the modular approach to building microfluidic network has been analysed theoretically. Other devices capable of generating such gradients usually require multiple inlets. And thus they have to make solutions with certain concentrations by diluting original solutions beforehand, or through a mixer module. To show the ability of our method in generating complex gradients, we design two convex profiles, both of which are described by two functions. By changing the flow resistances in the network or connecting ways of mixed solutions at the collector manually, we can dynamically transform one gradient to another. We believe our method provides not only a powerful alternative for gradient generation, but also an inspiration for designing other microfluidic control devices.

**Experimental**

**Theory**

To obtain a desired concentration gradient, our method is to combine fluidic streams at various flow rates into laminar flows that carry different concentrations (similar to ref. 15 and ref. 16). Apart from the modular structure, the difference our approach has compared with existing ones is that various flow rates in the system are adjustable in our approach and are achieved through modulating flow resistances using variable microfluidic resistor (we abbreviate it as VMR). Complete mixing is accomplished through diffusion in connecting tubes before converging into the functional section of the gradient generator for detection and analysis. Because of this design, our system is independent of flow rates in that mixing events through molecular diffusion only happen once for pre-mixed solutions.

For the purposes of our discussion in this work, fluidic circuits can be analogous to electrical circuits. In microfluidic channels, flows are generally laminar due to low Reynolds numbers. When neglecting hydraulic inertial behaviors such as liquid compressibility, a simple relationship among flow resistance $R$, pressure drop $\Delta P$ and flow rate $Q$ is expressed in eqn. (1), similar to Ohm’s law. For a rectangular channel with width $w$, height $h$ and length $L$, $R$ can be calculated according to eqn. (2), where $\mu$ is the fluid viscosity.

$$\Delta P = QR$$  \hspace{1cm} (1)

$$R = \frac{12\mu L}{wh} \left[1 - \frac{h}{w} \left(\frac{192}{\pi} \sum_{m=1}^{\infty} \frac{1}{n^2} \tanh \left(\frac{nw}{2h}\right)\right)\right]$$  \hspace{1cm} (2)

In an electrical circuit, various functional components modulate the distribution of electrical current, such as switches, sliding rheostats, capacitors. These components are modular and standardized to allow easy integration into circuits with complex functions. We designed several fluidic components that mimic the roles of some electric components (Fig. 1). They are used to distribute, regulate, combine and collect aqueous flows. All the modules have simple patterns, and can be fabricated by one-layer photolithography. Fig. 1(a)–(e) are the designed modules, and Fig. 1(f)–(j) illustrate the corresponding flow resistances. According to eqn. (2), when the height and the width of microfluidic channels are fixed, fluidic resistance is proportional to the channel length $L$, i.e., $R = cL$, where $c$ is the coefficient. In our design, distributors, VMRs, combiners and collectors have the same height and width (100 × 28 μm, except for Fig. 1(e)), so their resistances share the same $c$. We set $L = 2$ mm as unit value, that is, $R = 1$. (For convenience, we define $R$ here as dimensionless.) We can thus design flow resistances by designing the lengths of microchannels. Fig. 1(a) is a module containing eight identical distribution channels, each has a flow resistance $R$, (Fig. 1(f)). It can produce eight branch flows of equal flow rate. Fig. 1(b) is a combiner; it is used to join two solutions together. It is composed of five $R_s$ (Fig. 1(g)). Fig. 1(d) and Fig. 1(e) are intended for collecting final flows carrying desired concentrations to combine them.

![Fig. 1](image)

An illustration of the modules employed in the system. (a) the distributor (b) the combiner (c) the VMR (d)–(e) collectors. Apart from (e), microchannels in all modules are designed with the same width and height (100 μm, 28 μm). (f)–(j): Illustration of the flow resistances of the modules. For (h), the value between $h_{o}$ (short for hole_odd) and $h_{e}$ is 1 by definition, and 3 for between $h_{e}$ and $h_{o}$, and so forth. At the other side, we get 2, 4, 6 for $h_{o}$ (short for hole_even) and $h_{e}$, $h_{o}$ and $h_{e}$. The value between adjacent holes in the middle of the module (a and b) is 7. The value determined by any of two holes in the module can be described as $R_s = 7n + m$ ($n = 0, 1, 2, \ldots, m = 1, 2, 3, 4, 5, 6$). Collector 1(d) has eleven separate channels, with the same length (28 mm), thus has eleven parallel $R_s$. Collector 2(e) consists of ten identical narrow channels and one main wide channel. The narrow channels are 100 μm wide, while the main one is 900 μm. The distance from the beginning of each narrow channel to the end of main channel is 28 mm.
into a gradient. Fig. 1(d) has eleven parallel Rxs. Fig. 1(c)
the variable microfluidic resistor. In addition to modulating
microfluidic resistance, VMR has holes that connect to external
tubing, serving as multiple world-to-chip interfaces. Only two
holes are not plugged so that the actual resistance is determined
by the length of flow path between them. The resistance of VMR,
$R_v$, is variable depending on which of the two open holes is
chosen (Fig. 1(h)). In the figure, the distance between $h_1$ and $h_2$
is 2 mm, thus $h_2 \sim h_1$ has a resistance value of 1. We put $h_1$, $h_2$, $h_3$, $h_4$, $h_5$, at one end of the module, and $h_1$, $h_2$, $h_3$, $h_4$ at the other.
The value between two serial holes in the middle of the module
(e.g. $h_3$ and $h_5$, a and b) is 7. Consequently we get values of $7n + m_i$ ($n = 0, 1, 2, \ldots, m_i = 1, 3, 5$) or $7n + m_i$ ($m_i = 2, 4, 6$) from
either end of the module. Using this approach, we can set $R_v$ to
be any integral number within an interval $[1, 137]$ in our design.
It can be altered in real time by changing the plugging patterns.

We construct fluidic systems using the modules displayed
in Fig. 1 and illustrate the design principles with a schematic
representation of a gradient generator made from a flush of four
concentrations (Fig. 2(a)). In this system, modules are connected
by polyether (PE) tubes with macroscopic dimensions
(the inner diameter is 0.5 mm). We feed the original fluorescent
solution and diluent into inlets that are in the center of an
eight-distributor module, where flows are split into three equal
daughter flows. (The other five holes are plugged.) These
flows encounter the same resistance in the module because of
the symmetric architecture. Two of the six flows are directly
connected to the collector module, while the rest are combined
in two combiner modules. The VMR modules modulate the flow
rates of pre-combined flows. Finally the resulting solutions are
fed into the collector, where they are detected and analysed,
together with the two initial solutions.

To illustrate our method of calculation needed for building
the fluidic system, we pick out a sub-flow circuit (Fig. 2(b))
and draw it in an electrical analogy (Fig. 2(c)). This analogy
is valid when we assume that all flows are laminar, and we
neglect secondary flows in junctions and turns as well as those
induced by curvature.\textsuperscript{30} In addition, we assume that fluids are
incompressible and tubes are rigid so that compliance can be
ignored.\textsuperscript{31} In Fig. 2(c), modules are represented by electrical resistances
and junction nodes, connecting tubes are shown as electrical wires,
and inlets and outlets are shown as power supplies and grounding.
We can find relationships between currents and resistances in Fig. 2(c)
by applying Kirchhoff’s and Ohm’s laws:

$$R_i + R_{id}(x_i + x_4) + (R_i + R_2 + 2R_i)x_4 - (R_i + R_2)x_6 = 0 \quad (3)$$

In Fig. 2(a), we can pick out 4 sub-circuits and write the corresponding equations. The other three equations are:

$$\begin{array}{l}
(R_i + R_4)(x_1 + x_2) + (R_i + 2R_2)x_1 - (R_i + R_2)x_4 = 0 \\
(R_i + R_4)(x_1 + x_2) + (R_i + R_2 + 2R_2)x_1 - (R_i + R_2)x_4 = 0 \\
(R_i + R_4)(x_1 + x_2) + (R_i + 2R_2)x_1 - (R_i + R_2)x_4 = 0
\end{array} \quad (4)$$

Together with two boundary conditions (we set $x_a$ and $x_e$ in
the syringe pump),

$$\begin{cases}
x_1 + x_3 + x_5 = x_6 \\
x_2 + x_3 + x_5 = x_6
\end{cases} \quad (5)$$

We thus have six equations, and six unknown branch flow rates.
By solving the equations, we can acquire the analytical
solutions of the six flow rates (assuming $R_i$ and $R_2$ are given).
Once branch flow rates are known, the output of concentrations
at the collector is consequently determined.

Now, we are able to obtain targeted gradient by setting
appropriate VMR values. To find the connections and values of
VMRs, like solving the black box problem, we need a few more
equations. In our system, the number of VMRs and combiners
are the same. By adding proportions of pre-mixed branch flows
as boundary conditions (i.e. desired concentrations, see below)
we again make the number of equations and unknown variables
the same. Solving equations provides values of both branch flow
rates and VMRs. The equations are nonlinear and the work is
done on Mathematica 5.0 and Matlab 7.0.\textsuperscript{34,35}

\section*{Fabrication of modules}

All the modules in our experiment were fabricated \textit{via} soft
lithography.\textsuperscript{36} Briefly, we designed the patterns using L-Edit
(Tanner EDA) and printed them on a commercial printer (www.
bjflfd.com). Then we made the master by contact photolithog-
raphy using SU-8 photoresist (MicroChem, Newton, MA).
We made all the patterns (in the form of SU-8 photoresist)
on a 4 inch silicon wafer to ensure a uniform height. After
fabrication in the clean room, we transferred the patterns
to poly(dimethylsiloxane) (PDMS) by replica molding. Then
we cut the PDMS replica into slabs carrying the appropriate patterns and drilled holes for interconnections using a needle with an inner diameter of 0.5 mm. The side of the PDMS slab with embossed channels and a glass slide were oxidized in an air plasma cleaner (200–600 mTorr, 2 min 30 s, 29.6 w). After oxidation treatment, we immediately assembled the PDMS slab and the glass slide into individual chips irreversibly. We used PE tubes as connecting media for modules to construct the system. The two PE tubes linking the two inlets of distributors were connected to syringes to provide constant working fluids.

Microscopy and measurements

We used fluorescein sodium in distilled water as the working fluids. The concentration of the fluorescent solution was 12 mg L$^{-1}$, which fell in the range of linear dependence of fluorescence intensities on concentrations. By using a digital monochromer camera (Leica DFC350 FX), we took fluorescence images with exposure time of about 400 ms. To minimize deviations of fluorescence intensity generated during joining partial images into complete images as well as inhomogeneous UV illumination, we calibrated the fluorescence intensity in the working channels using a standard method. Data were analysed on the software provided by Leica (LAS AF). For the collector 1, we drew a rectangle to cover as much area as possible in each channel and calculate the average intensity in this area. While for collector 2, areas for measuring average fluorescence intensities were selected on narrow channels before merging into the wide channel to minimize noise from adjacent flows. To normalize data, we first treated intensities at the diluent channel as background intensities, and subtracted it from other intensities. Then we divided them by the maximum value so as to set the undiluted solutions to be 100% in concentration. All data fell between the interval [0 1].

Results and discussion

System design and calculation

The model system in our experiment consisted of two-one-eight distributor modules ($R_i$ in the Fig. 2), seven VMRs ($R_{12}$ to $R_{17}$), seven combiners ($R_i$), two different collectors ($R_i$) and PE tubes. The sixteen branch flow rates $x_{1-10}$ from the two distributors to the combiners were arranged as follows: $x_1$, $x_1$...$x_{15}$ were the diluents and $x_2$, $x_4$...$x_{16}$ were the original fluorescent solutions; $x_2$ and $x_3$, $x_4$ and $x_{13}$ and $x_14$ were combined in the seven combiners while $x_{15}$ and $x_{16}$ were directly connected to the collector, resulting in nine channels carrying different concentrations. The order of the nine flows at the collector was $[x_{15}$, $x_1 + x_2$, $x_3 + x_4$, $x_5 + x_6$, $x_{13} + x_8$, $x_{11} + x_{12}$, $x_{14} + x_{16}$], and the corresponding concentrations were:

$$\begin{align*}
0, \frac{x_2}{x_2 + x_3}, \frac{x_4}{x_4 + x_5}, \frac{x_6}{x_6 + x_8}, \frac{x_7}{x_7 + x_9}, \frac{x_8}{x_8 + x_9}, \frac{x_{10}}{x_{10} + x_{11}}, \frac{x_{11}}{x_{11} + x_{12}}, \frac{x_{12}}{x_{12} + x_{13}}
\end{align*}$$

The concentrations we use here were based on volumetric flow rates, thus were dimensionless. This system can be quickly built and allows real-time plug-and-play. Some modules are flexible in function. For example, the one-eight distributor can be a one-$n$ distributor ($n \leq 8$) when unused holes are plugged. For the VMR, the designed upper limit value of flow resistance is 137; we could get even larger values by connecting two or more in series.

A syringe pump drove two starting flows. The outlet of the system was at the exits of the collectors. They were under atmospheric pressure. We treated these exposed outlets as grounded so as to finish the circuit loops. $R_i$ and $R_{18}$ were set to 2 and 1.5, and $R_1$ was 14. We ignored flow resistance contributed by PE tubes. This is reasonable because the tube we used had an inner diameter of 0.5 mm, resulting in a much larger cross sectional area (2.0 × 10$^{-5}$ m$^2$) than the microchannels (2.8 × 10$^{-5}$ m$^2$). Now based on conditions described above, we can write out the general form of the equations that describe the system mathematically:

\[
\begin{align*}
\left( R_3 + R_1 \right) (x_{13} + x_{14}) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0; \\
\left( R_3 + R_1 \right) (x_{14} + x_{15}) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0; \\
\left( R_3 + R_1 \right) (x_{15} + x_{16}) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0; \\
\left( R_3 + R_1 \right) (x_1 + x_2) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0; \\
\left( R_3 + R_1 \right) (x_3 + x_4) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0; \\
\left( R_3 + R_1 \right) (x_5 + x_6) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0; \\
\left( R_3 + R_1 \right) (x_7 + x_8) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0; \\
\left( R_3 + R_1 \right) (x_9 + x_{10}) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0; \\
\left( R_3 + R_1 \right) (x_{11} + x_{12}) + \left( R_3 + 2R_3 + R_{12} \right) x_{13} - \left( R_1 + R_1 \right) x_{10} = 0;
\end{align*}
\]

(6)

\[
\begin{align*}
x_1 + x_5 + x_7 + x_9 + x_{11} + x_{13} + x_{15} = x_\gamma, \\
x_2 + x_4 + x_6 + x_{10} + x_{12} + x_{14} + x_{16} = x_\delta;
\end{align*}
\]

(7)

\[
\begin{align*}
x_2 = c_1; \\
x_4 = c_2; \\
x_6 = c_3; \\
x_8 = c_4; \\
x_{10} = c_5; \\
x_{12} = c_6; \\
x_{14} = c_7; \\
x_{16} = c_8.
\end{align*}
\]

(8)

$R_{12}/i (i = 1...7)$ and $0/R_{18}$ in eqn (6) are used since positions of $R_{18}$ are not determined. (e.g. $R_{18}$ in Fig. 2(a) is connected in $x_{16}$, thus should appear as a factor before $x_{16}$, while before $x_{16}$ is 0). Eqn (8) contains the boundary conditions that describe the relationships between branch flow rates and thus the desired profiles. In order to show the all-purpose property of our system,
we designed two relatively complex profiles (Fig. 3). Fig. 3(a) comprises a rising line (left) and an exponentially decaying curve (right). Fig. 3(b) comprises a parabolic curve and a line.

Before we did the calculation, we had to assume the positions of VMRs first. First we connected them in even branches (i.e. \( x_2, x_4, x_6, x_8, x_{10}, x_{12} \) and \( x_{14} \)) so that \( R_{2-1}/0 \) became \( R_{2-2} \), for the former seven equations, while 0 for the latter seven equations in eqn. 6. Then we solved the equations in the software and saw if the symbols of the values of VMRs were positive. If not, we placed those having negative values in odd branches and calculated the modified equations again. For the first profile, we set \( x_o = 6 \text{ ml h}^{-1} \), and \( x_e = 4 \text{ ml h}^{-1} \). The calculated results were that \( R_{2-1} \) to \( R_{2-7} \) were placed in \( x_2, x_4, x_6, x_8, x_{10}, x_{12} \) and \( x_{14} \) branches, and the values were [26.9858, 7.94141, 1.59328, 12.7025, 37.9805, 65.6889, 84.119]. Since they were beyond the resolution of VMR (i.e. 1), we rounded them into integral numbers: [27, 8, 2, 13, 38, 66, 84]. For Fig. 3(b), we set \( x_o = 4 \text{ ml h}^{-1} \), and \( x_e = 5 \text{ ml h}^{-1} \). We got [2, 20, 131, 25, 7, 1, 6]. And \( R_{2-1} \) to \( R_{2-7} \) were placed in \( x_1, x_3, x_5, x_7, x_9, x_{11} \) and \( x_{13} \). Using the after-rounding data, we got the corresponding flow rates \( x_1-x_{16} \) and thus the gradient profiles, and plotted them against the before-rounding profiles (original profiles described in the previous paragraph) in Fig. 3. We can see that the rounding of data hardly changes the profiles. The rounded values were adopted to set the values of VMRs and thus the after-rounding profiles were used as theoretical references.

Results of the experiment

1 Generation of stepwise gradients

To test the accuracy of the profiles and their stability at different flow rates, we set the total flow rate (i.e. \( x_o + x_e \)) to be 2, 4, 6, 8, 10 ml h\(^{-1}\) for the desired profile in Fig. 3(a) and 1.8, 3.6, 5.4, 7.2, 9 ml h\(^{-1}\) for the desired profile in Fig. 3(b). The averaged data from the five flow rates are plotted with theoretical data in Fig. 4. Standard deviations for each concentration are also shown as error bars. The maximum standard deviation was ±0.059 and the minimum was ±0.003 among Fig. 4(a) and (b). As for the
first channel and the fifth channel in both the two profiles, the standard deviations were zero because the concentrations were set to be 0 and 100% during normalization. We can see that experimental data are in good agreement with theoretical values, and results are not affected by various flow rates.

To generate continuous gradients inside a single stream, we replaced collector 1 with collector 2. In collector 2, nine individual flows were combined in the main channel. The main channel had a width of 900 \mu m (this is because we use nine narrow channels). The path between each inlet at the narrow channel to the outlet at the main channel was equal to the length of each individual channel in collector 1 (Fig. 1). In this sense, it seems that collector 2 could be treated as equivalent to collector 1 in terms of resistance. The resulting profiles, however, had some deviations, especially for the first profile in Fig. 4(c). In Fig. 4(c), the values of experimental data were all obviously smaller than that in Fig. 4(a). As for Fig. 4(d), most data were larger instead. Actually, the flow resistance of collector 2 was not equivalent to collector 1. In collector 2, most individual streams after combination were neighbored by other streams, leading to a reduction of friction compared to collector 1, where flows were sheathed by PDMS walls. As a result, the resulting resistance was smaller than collector 1. We theoretically calculated the gradient profiles in response to different values of the collector \( R_4 \) (Fig. 5(a), (b)). The results showed that with the decrease of \( R_4 \), values of concentrations decreased for the first profile (from Fig. 4(a) to Fig. 4(c)) and increased for the second one (from Fig. 4(b) to Fig. 4(d)). And the changes in the first profile were more sensitive to the changes of the values of \( R_4 \) than those in the second one.

In order to increase the accuracy in the profiles of gradients in collector 2, we added a collector 1 in the circuit upstream of collector 2. This operation made the equivalent resistance larger in the system. As a result, we can see that experimental data are closer to theoretical ones (Fig. 5(c), (d)). We do not have to figure out the exact resistance of collector 1 + collector 2, because according to theoretical calculation, the profiles do not change much when \( R_4 > 14 \) (Fig. 5(a), (b)).

2 Altering gradient profiles in real time

The gradient profiles are determined by systematic flow resistances, mainly VMRs. Thus, we can in real time change the profile by changing VMRs, including their values and positions. This was a convenient operation, which involved pulling out or inserting the PE tubes into the VMRs and adjusting their connectivity. We demonstrated this capability by changing the output from the first profile to the second. In Fig. 6, when the first profile became stable in the system at a total flow rate of 2 ml h\(^{-1}\), we quickly changed the values of VMRs and corresponding connections, and set the total flow rate to be 9 ml h\(^{-1}\). After that, we captured the fluorescent images every two minutes. Sixteen
Switching between two types of profiles by adjusting VMRs and flow rates. The intensities were measured every two minutes. The profile kept almost unchanged from 20 min to 40 min until the flow in the slowest channel reached the collector.

minutes later, the profile was almost shifted to the second shape. The final shape appeared at 42 min after the switch. The response was slow because the flow rate in the premixed branch that lead to the fourth concentration in the second profile (theoretical value 0.951) was rather slow. In fact, in other cases where the connections of the two profiles are the same, we only need to change values of VMRs, a total cost of time would be less than 8 min for the current system. This dynamic property is helpful in especially temporal and spatial control of cell chemotaxis, since it provides a well-controlled environment to study, e.g., directional sensing of polarized cells to signaling molecules.

To generate new profiles, we can recalculate the requirements in flow resistance in the system to obtain another set of information about VMRs, or we can just permute the order of channels before connecting to the inlets of the collector. Fig. 7 is a demonstration that we can convert a convex profile (outlined in Fig. 4(d)) to a concave profile directly. Here, the profiles switched immediately after we relocated the PE tubes at the inlets of collector 2, unlike what is described in Fig. 6, which developed gradually. It thus provides a fast method for switching between certain types of gradients.

**Advantages of modular approach**

We believe that as a gradient generator, microfluidic systems based on modular architecture are powerful in producing complex and tunable gradient profiles. Other systems can hardly achieve this function due to their solidified and integral structures. In the modular approach, key components are fabricated in forms of separate modules and consequently combined flexibly. The combination can be done before and during the operation of the system, which enables control in real time and ensures that the system can work continuously when local damage happens. Changing modules with fresh ones can also help preventing contamination. When experiments are done, the system can be disintegrated into modules that can be stored and reused. Each new design of gradient does not automatically call for a new process of microfabrication. Doing so will reduce the cost and time of experiments, particularly for the rapid prototyping of the responses of biological cells to different types of gradients.

More importantly, modular approaches such as the one described here might provide access to microfluidic devices to potential users otherwise poorly equipped to fabricate microdevices. Taking our system for example, it employs readily available techniques such as replica molding to generate all basic components, once the masters are made. With the masters for a few basic components, any type of gradient can be generated by combining and connecting these basic components, without the need for further microfabrication. Doing so will provide gradients of many types to users with no resources for microfabrication, such as most cell biologists.

Our system may be easily upgraded with new functions. For example, if we put a clamp on the PE tubes, modules downstream will be cut off from flows. This macroscopic valve is convenient to operate and obtain compared to microvalves integrated in channels. As for the VMR module, it may also find potential uses in measuring hydraulic pressure drop, or flow resistance. It may be used to determine equivalent flow resistance of channels with irregular bas-relief structures on their floors, which cannot be directly calculated from eqn. (2). Apart from generating gradients in solutions, we can also generate complex gradients on surfaces, if combined with microcontact printing. In this regard, the system is open to the incorporation of new functional components and is upgradeable.

**Disadvantages of our system and further improvements**

The major disadvantage of our system is the large overall volume of solutions required. Since the tubes have an inner diameter of 0.5 mm, the overall volume of fluids compared with systems on single chips is rather large. Besides, we employ PE tubes as interconnections, if more modules are involved, more tubes will be used. As a result, our system may appear quite complex with many tubes intertwining over the modules. To solve this problem, we can choose soft capillaries with much smaller diameters, or
make them inside a board, similar to the idea of microfluidic breadboards. In those cases, flow resistances of connecting channels shall have to be taken into careful consideration. They shall be calculated or measured before added into equations. At present, this system employing PE tubes is therefore only applicable to experiments that do not require small amounts of fluids.

Manual handling for changing gradient profiles or replacing modules is a feature of our system. Since PDMS is a soft material, flows in microchannels can be disturbed when we put in or pull out plugs on VMRs. Doing so could cause the gradient to be unstable for a few minutes. But if we finish the operation within seconds, the stability of the gradient is unaffected.

Further improvements to our system are possible. The combiner (Fig. 1(b)) can be replaced by the distributor, as long as six of the holes are plugged. We have retained the combiner to make easier the comparison of our work to some of the components described theoretically in ref. 30. For further application, a more compact system based on three modules (distributor, VMR and collector) could be capable of complex gradient generation. Second, from Fig. 5(a), (b) we see that profiles are less sensitive to changes of flow resistances in the system when the resistances are large. We can, therefore, design modules with large resistances to make profiles stable against changes of local resistances caused by imperfections in the fabrication process. Third, we can add more VMRs in the branch circuits to ensure that nine flows in the collector have the same flow rate. It can be realized by providing more boundary conditions and thus solving more equations to get the information about the added VMRs.

Conclusions
We have demonstrated a modular approach to generating arbitrary gradients on the micrometer scale spatially and temporally. Based on the analogy between electrical and fluidic circuits, we present a practical method for building the system using simple modules. Using the system, we can produce complex gradients that can be potentially used in simulating various situations in vivo. This system will likely broaden the biological applications of microfluidic channels by providing gradients to users who do not have access to microfabrication. In addition, this system can also be a good model system for scientists interested in understanding the fundamental behaviors of fluid flows at micrometer-sized spaces in an integrated fluidic system.

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