Modularized Concentration Gradient Generator

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Abstract

The purpose of Modularized Concentration Gradient Generator (MCG) is to develop various gradient combinations by adding or removing modules. With 1 main module and 4 branch modules as described in the Figure 1, MCG can generate 17 different concentrations. Each module has 2 inlets and 5 outlets with circular shape. By using circular shape, the device can be made in the optimized area compared to the conventional concentration gradient generators which have long designs. Also, in order to give solutions enough time for diffusion to be occurred, the design has serpentine channel structures. The experiments were developed into two steps, to simulate the device by using COMSOL Multiphysics® and to fabricate the device by soft lithography.

1. Introduction

Making concentration gradients of diffusible solution is important in many fields such as biological research such as Minimum Inhibitory Concentration (MIC). [1] Recently, in order to do better research, concentration gradient generator using microfluidic device has been developed. [2] Concentration gradient generator is a microfluidic structure that creates diverse concentration by simply flowing different solutions into its inlets. There are diverse concentration generators e.g., 2D structure that creates concentration gradient purely by diffusion, constantly diluting the solution by introducing buffer as the solution flows by etc. [3], [4], [5] However, none of them seem to have efficient way of creating and sorting diverse and detailed concentration gradients. In short, to find more various concentration gradient combinations with less labor, new concentration gradient generator which is easy to modify is required.

In order to meet those needs, the new concentration gradient generator, Modularized Concentration Gradient Generator (MCG) is proposed. MCG is radial-shaped concentration generator with a lot of meandering structures. By making the device radial-shaped, it is easy to divide the device into modules. Meandering structures give solutions enough time to be diffused properly. Also, because it is modularized design, it is easy to add or remove modules. This paper will mainly handle basic MCG design which has 1 main module and 4 branch modules as described in the Figure 1.

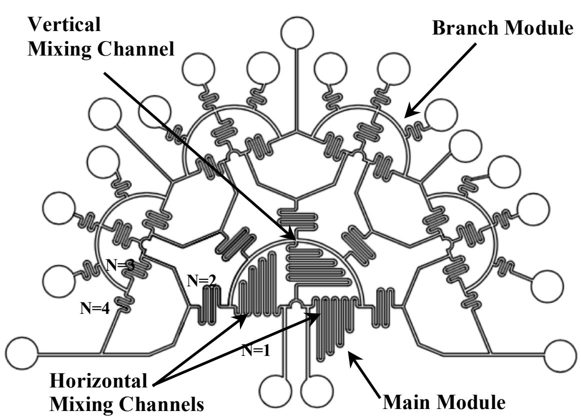


Figure 1 *Full Design of Modularized Concentration Gradient Generator. There are 17 outlets and two inlets. Also there are 1 main module and 4 branch modules*

2. Design strategy

2.1 Device design

There are multi-circle channels and serpentine mixing channels in radial concentration generator. Two inlets (2mm diameter circular holes) are connected to the main module and the connection channel is 200 ㎛ width and

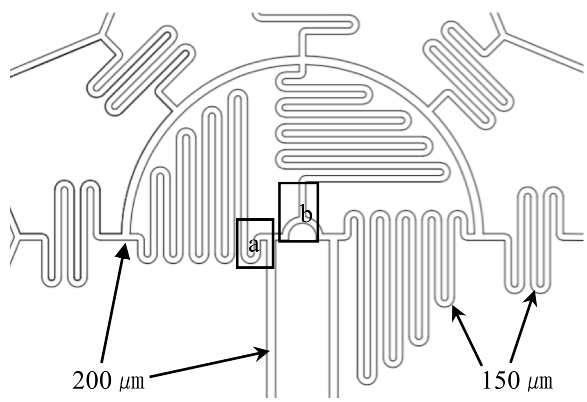


Figure 2 *Specific Design strategies. For the mixing channel, channel width is 150 ㎛ and for the non-mixing channel, channel width is 200 ㎛.a and b indicates the length compensation.*

Table 1 *Channel Length – Minimum requirements for the length* = $27000/2^{N-1}\ \mu m$

N	**1**	**2**	**3**	**4**
Minimum Requirements	27000 μm	13500 μm	6750 μm	3375 μm
Actual Channel Length	27631.15 μm	14995.36 μm	6824.76 μm	4004.46 μm

100 μm height. Also, the outermost circular channel of the main module is 200 μm width and 100 μm height. These are for the non-mixing part where the mixing operation does not occur. For the mixing parts which are composed of serpentine channels, have 150 μm width and 100 μm height. The lengths of the channels are based on the equation as follows. [6]

$$Length = \frac{27000}{2^{N-1}}\ \mu m \tag{2.1}$$

N value of the equation (2.1) is channel number which is described on the Figure 1. This is the minimum requirements for the length value because if the channel lengths become longer, diffusion can occur more completely. The actual values and minimum requirements for the channel lengths are described on the Table 1. Also, in order to make channel lengths exact same, length compensation is applied. In Figure 2, there is a difference between a part and b part. This is because the distance between inputs and mixing channels are different. In Figure 2, distance between input and horizontal mixing channel is shorter than distance between input and vertical mixing channel. In order to compensate this difference, a part of Figure 2 is applied so that the all lengths of mixing channels are same.

2.2 Fabrications

The structure described on the Figure 3 was fabricated by PolyDiMethylSiloxane (PDMS) using soft lithography and the wafer was fabricated by photolithography. In brief, photo curable polymer SU8 was spin-coated on a wafer. After soft baking, it was inserted with a mask in a photolithography device, and exposed to Ultra Violet (UV) light. Post exposure bake was carried out after the exposure, and then the wafer was rinsed by thinner. With this fabricated wafer, the device can be prepared by pouring the PDMS onto the master. The hardened PDMS bonds permanently to the glass substrate after activated in the plasma etcher.

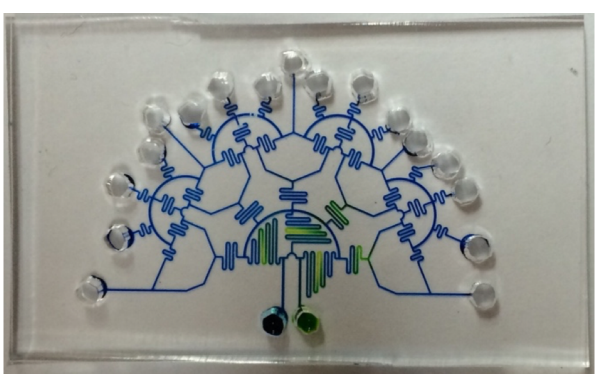


Figure 3 *Fabricated Design by soft lithography.*

3. Simulations

To simulate the device, COMSOL Multiphysics® was used in this paper. The conditions for the simulation is on the Table 2. In the simulation, 'Laminar Flow' is applied first and then 'Transport of Diluted Species' is applied next. This is because, in order to simulate properly, the channels should be filled with laminar flow before applying diluted species. After filled with laminar flow, diluted species are applied in order to visualize the result.

The result of the simulation is on the Figure 4. If 0 mol/m^3 is applied to one inlet and 100 mol/m^3 is

Table 2 *Conditions for the simulation using COMSOL Multiphysics®*

Conditions	**Parameters**
Temperature	20 °C
Diffusion Coefficient	2.022e-9 *
Channel Material	Water
Non-Channel Material	PDMS
Laminar Flow - Wall Condition	No-Slip Wall
Laminar Flow - Inlet Pressure	1000 Pa
Laminar Flow - Outlet Pressure	0 Pa
Laminar Flow - Study Model	Stationary
Transport of Diluted Species – Transport Properties	Velocity Field as a Result from Laminar Flow
Transport of Diluted Species – Initial Value	Concentration = 0
Transport of Diluted Species - Inflow	Inlet 1 - 100 mol/m^3 Inlet 2 – 0 mol/m^3
Mesh	User-Controlled Mesh
Study	Stationary Step

* Self Diffusion Coefficient of Water at 20 °C

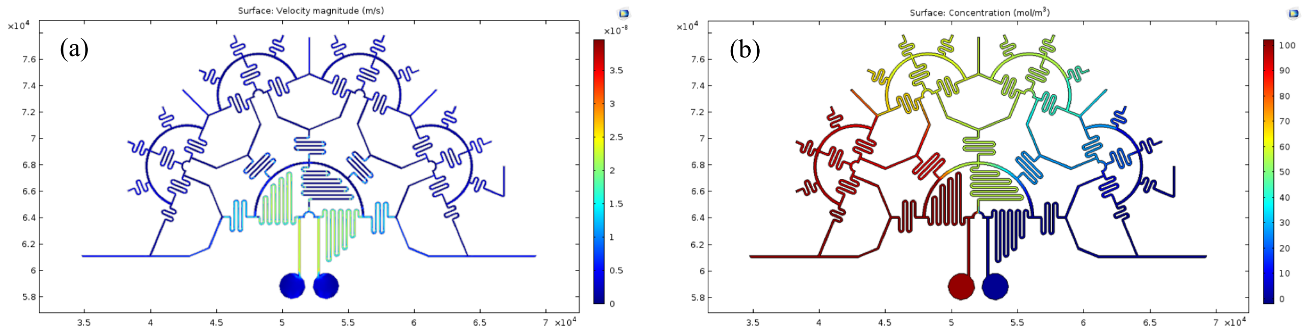


Figure 4 *(a) Surface Velocity Magnitude (m/s) of the device. In the main module, there is difference between mixing channels. (b) Surface Concentration (mol/m^3) of the device. In the 45-degree and 135-degree of the main module, two different species (red-yellow & yellow-blue) are misaligned. It should be half-half.*

applied to the other inlet, the expected outlet concentration should be the values listed on Table 3. However, the simulation result was not as expected. This result is due to the misalignment of two species on the main module. In Figure 4b, 45-degree and 135-degree of the main module should be half and half of the two species, but the simulation result was not as expected. This result can be explained by surface velocity magnitude on the Figure 4a. In the main module of Figure 4a, there is a difference between horizontal mixing channel and vertical mixing channels. By using color scaling bar on the right of the Figure 4a, it can be expected that the velocity of the vertical mixing channel is slower than the horizontal mixing channels. This is because inlets are closer to horizontal mixing channels than the vertical one. Even though the lengths of the channels are the same, flow velocity is influenced by how close inlets and mixing channels are. For vertical mixing channels, flow should pass the quarter of the small circle in the very middle part of the design as described on Figure 2b, so the velocity of the flow is decreased. The flow of horizontal mixing channel is faster than the vertical one, so on the 45-degree and 135-degree of the main module, solutions from the horizontal mixing channels which are red and blue in Figure 4b is dominant. This was the main reason for the failure of simulation.

Table 3 *Expected outlet concentration and simulated result (Unit = mol/m^3)*

Outlet No.	Expected Values	Simulated Values
1	100	99.99895076
2	93.75	97.43971545
3	87.5	95.55420752
4	81.25	93.35113699
5	75	80.17052065
6	68.75	65.50645013
7	62.5	60.37039183
8	56.25	59.82597159
9	50	58.15007537
10	43.75	56.35583808
11	37.5	55.56252333
12	31.25	43.37590261
13	25	33.75432296
14	18.75	16.84793498
15	12.5	4.644812822
16	6.25	1.280477847
17	0	0.001793839

**Outlet Numbers are counted from right corner to the counterclockwise direction

4. Experimental Results

On this paper, two experiments were proceeded, dye experiment and FluoresceinIsoThioCyanate (FITC) experiment. Dye experiment is for visualization of the overall result, and FITC experiment is for numerical analysis.

4.1 Dye Experiment

For the dye experiment, yellow and blue color is used so in the middle part of the device, green will appear. In Figure 5a, it seems to be proper result that the left side of the device is blue where as right side is yellow. Also middle part of the device is green. The reason that the

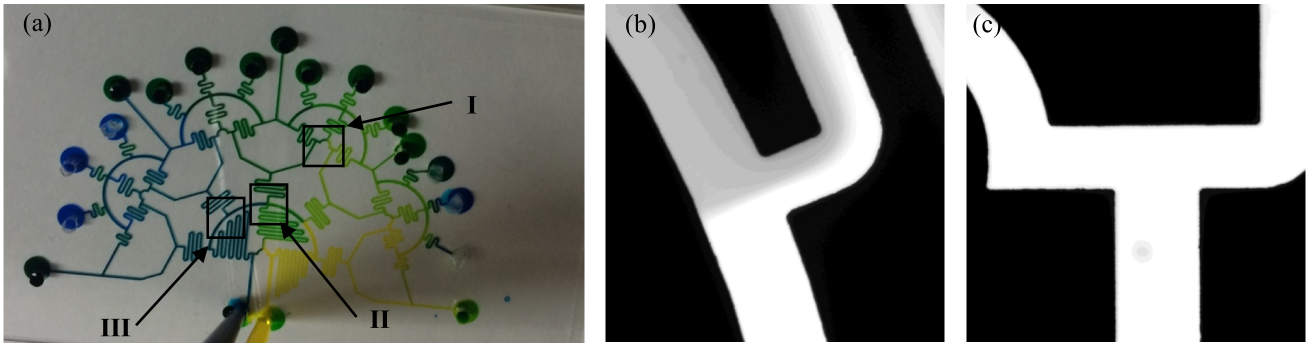
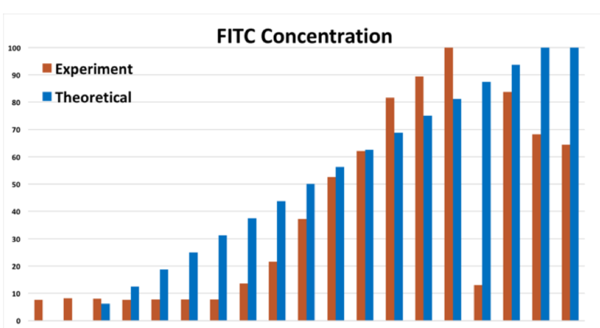


__Figure 5__ (a) The result of the dye experiment. (b) The result of FITC experiment where two different concentrations meet together and flow to the mixing channel. (c) The result of FITC experiment on inlet.

outlets are dark is because before two inlets are applied, blue dye was applied first in order to fill the device with laminar flow. After filled with blue dye perfectly, yellow dye was applied. However, the result was not as expected. Although in some joints where two different dyes meet worked properly as Figure 5a-I, other joints were not worked exactly. In Figure 5a-II, it should be pure green theoretically, but the result shows that it is the mixture of green and blue. Also, in Figure 5a-III, it should be the mixture of green and blue theoretically, but it was just pure blue. This result is similar to the simulation. Due to flow velocity, pure blue flow seems to be more dominant than green flow in Figure 5a-III. Moreover, pressure was controlled with height of the solution, not the syringe pump, so the pressure cannot be perfectly same. Also, in the experiment, pipets were not perfectly fitted with the inlets so there was some leakage between the gap. This gap has the possibility of making pressure differences inside the channels.

4.2 FITC Experiment

FluoresceinIsoThioCyanate, which stands for FITC is fluorescent solution which can be seen clearly under UV light. By measuring the brightness of it, numerical analysis can be examined.



** For the FITC experiment, pure water is applied to the left inlet and FITC solution is applied to the right inlet, which is opposite from simulation. Y-axis indicates the percentage.

__Figure 6__ Graph of FITC experiment

For the FITC test, pure water is applied to the left inlet and FITC solution is applied to the right inlet, which is opposite from simulation. The picture of FITC experiment is on Figure 5b and 5c. In Figure 5b, two different solutions have met in the joint channel and flow to the mixing channel. Figure 5c indicates right inlet channel where pure FITC is applied, which is brightest among all.

The result of FITC experiment is graphed on Figure 6. Blue bar is expected concentration of the experiment and red bar is experimental results. As expressed on the Figure 6, the experimental result was not as expected. Outlet number 15 has much less concentration than others and outlet number 12, 13 and 14 has high concentration rate than pure dye which is abnormal. There are three reasons for the result. First, there were some bubbles inside the channels. There was a bubble inside the channel which is connected to the outlet number 15. Due to this, FITC solution was not flowed properly to the channel. Second, pressure was controlled with height of the solution, so the pressure cannot be perfectly even. Third, before applying FITC solution, pure water was applied first. After filled with pure water, FITC solution was applied. Due to this process, water was mixed with the solution in the FITC inlet (right inlet) first. If there were no bubbles inside the channels, the flows would go properly and each flows would find their own channels as time goes. However, there were some bubbles inside the channels so the mixed solution of water and FITC on FITC inlet (right inlet) could not escape. This is the reason for the abnormal concentration on outlet number 12, 13 and 14.

5. Conclusion

In this paper, a new concentration gradient generator which is Modularized Concentration Gradient Generator (MCG) is proposed. MCG is composed of modules so that it can be easily added or removed in the design. In this paper, basic MCG design, which has 1 main module and 4 branch modules are mainly handled. The basic MCG can generate 17 different concentrations. To test the performance of the device, simulation with COMSOL Multiphysics®, experiment with dye and experiment with FITC were proceeded. However, the results of the tests were not as expected. There are several reasons for this failure. First, pressure was driven by height of the solutions. Second, bubbles were formed in the channels in FITC test. Third, there were some gaps between pipet and inlet in the dye experiment. Due to this, the pressure was not driven properly. Finally, in order to make the design performs properly as expected, simulation should proceed first and then revision of the design should be go on until the simulating performance meets the expectation. Fabrication should proceed after this process. However, due to time limitation, fabrication was proceeded with simulation. First two problems can be solved by using syringe pump instead of pipet. The gap problem can be solved by sealing the inlet and pipet using PDMS. Finally, in order to solve the final problem, sufficient simulation and edition should be proceeded for making the device.

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