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# Fabrication of the PDMS microchip for serially diluting sample with buffer

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**Abstract** We developed a polymer polydimethylsiloxane (PDMS) based microfluidic device that dilutes biological samples with buffer solutions with serially increasing diluted sample concentrations. The device showed the relatively high accuracy in terms of dilution ratios along with the fact that it was faster and easier to operate. With two simple disposable plastic syringes, the plastic microfluidic chip completes ordinary sample dilution sequences faster and more precisely than the conventional manual pipette process in biological or chemical laboratory. The serially diluting mechanism of the microchip is simply that the number of microchannels with the same flow rate determines the total amount of flow into the wells. The soft lithography fabricated the microchannels of tetragonal section length of 50 μm of each side of the microfluidic chip.

## 1 Introduction

In biological or chemical laboratories, liquid sample or reagent preparation with various concentrations – which is referred to as 'serial dilution' in this paper – is an essential process applied in many experiments. But in general, this process can only be done with relatively tedious manual pipette processes whose tolerance depends almost on the skill of the researcher handling the pipette tools with sample solutions in it. Moreover the conventional serial dilution is a kind of time-consuming process owing to its critical inefficiency in its procedure. For example when diluting biological or chemical sample solution with buffer solution into 96-well-plate with different sample concentrations in each well, there are 192 times of pipette regulation, and 384 times of solution suction and infusion. And for the precise concentration control, all the plastic pipette tips have to be disposed of after infusing solution to wells, and those are 96 pipette tips in total. In spite of the simplicity in theory of the whole process, it's a drastically laborious job for the researcher who has to do that everyday.

These problems have already been considered, and some devices using microfluidic technologies were presented [1–3]. But their basic concepts generally involve microscale mixing by simple diffusion [4] which has many drawbacks in its realization. Basically flow generated in microscale channels is laminar where eddies that primarily invoke microscale mixing do not exist, so microscale mixing does not occur easily [5]. That is the main reason that present microscale mixing approaches induce time-dependant diffusion between flow layers [6] or use 3 dimensional complex microchannel features [7] or attach additional instruments [8] to make microscale mixing occur with in a short time.

But on the contrary in microscale flow, the laminar characteristics make it easier to design and control the streamlines without generating any undesired mixing and brings about exact flow distribution and flow splitting possible with higher accuracy. So we designed the serial dilution concept into two subdivisions; First, main inlet (sample and buffer) flow into destined numbers of subchannels that decides sample to buffer mixing ratios and second, summing up the channels and mixing them in a macro-scale well chambers together. To accomplish the first goal, we adopted microchannels whose laminar flow characteristics ensure equal distribution with each channels, and for the second goal we simply abandoned microscale mixing and allowed the mixing to occur in relatively huge well chambers with which miscellaneous micromixing troubles banish because it is not a microfluidic phenomenon.

By merely giving up the microscale mixing and making it occur by itself in macro-scale well chambers instead, we're able to accomplish a reliable and robust serial dilution.

## 2 Fabrication

The serial dilution microchip device was fabricated in poly(dimethylsiloxane) (PDMS) using soft lithography [9] as is shown in Fig. 1. The choice of the material PDMS depends primarily on its biocompatibility because this

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device is designed to dilute biological samples [10]. Briefly, CAD file drawing has been patterned on a glass substrate with chrome masking. The chrome mask was used in 1:1 contact photolithography with SU-8 photoresist to generate a negative master mold, consisting of patterned photoresist on a Si wafer. Positive replicas with embossed channels were fabricated by molding PDMS against the master [11]. Two inlet ports (2-mm-diameter holes) for the fluids were punched out of the PDMS using a simple steel hand-puncher. The surface of the PDMS replica and a clean slide glass were activated in an oxygen plasma (2.666 $\times10^{-2}$ Pa, 20 s, 25 W) and was brought together immediately after activation. An irreversible seal was formed between the PDMS and the slide glass [11]. Finally, this assembly produced the required systems of microfluidic channels.



## 3
## Results and discussion

The fabricated microchip is slightly smaller than a slide glass (60 mm × 20 mm × 3 mm, in width, height and thickness) and has 8 chambers that finally contain serially diluted samples with buffer solution (Fig. 2a). Two plastic syringes each containing the sample solution and buffer whose needles have been substituted by polyethylene tubing with outer diameter slightly larger than the inner diameter of the port was inserted into the two inlet holes to make the fluidic connections (Fig. 2b). And both the syringes were manually and independently pushed to inject each solution into the fluidic networks.

Main channel connected to the inlet port has a width of 900 μm and consequently splits into 9 subchannels of 50 μm in width (Fig. 3a). Serially aligning these 9 subchannels with linearly increasing numbers (Table 1) ensures the 8 serially increasing different concentration distribution with the accurate integer-to-integer ratio. For example, for the well chamber on the left end as in Fig. 2a, there is one channel that came from the sample port and 8 channels from the buffer port, and with this channel configuration sample is mixed with buffer of 8 times in quantity which makes the concentration of the original sample to be 1 over 9 of the initial value; the channel configuration is shown enlarged in the Fig. 3b. Similarly for the well chamber at the right end, there are 8 channels for the sample and one channe l for the sample, which results in the generation of 8 over 9 times weaker concentration of the initial value. Between these two, there are linearly increasing sample concentrations ranging from 1 over 9 to 8 over 9 of initial value in each well chamber.

With this algorithm, we could achieve serially increasing dilution concentrations as is verified in Fig. 4. Moreover with this concept, we can design variable flow networks that generate some desired concentrations for certain kinds of experiment protocols by rearranging sample and buffer channels on the microchips. We can add the final well chambers for more numbers of concentration variations and can also make more inlet ports to mix three or more samples in a complex configurations.

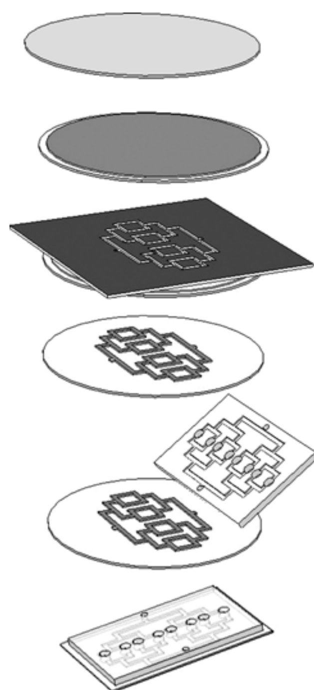


**Fig. 1.** Scheme describing the steps involved in the fabrication process of PDMS-glass hybrid microchip

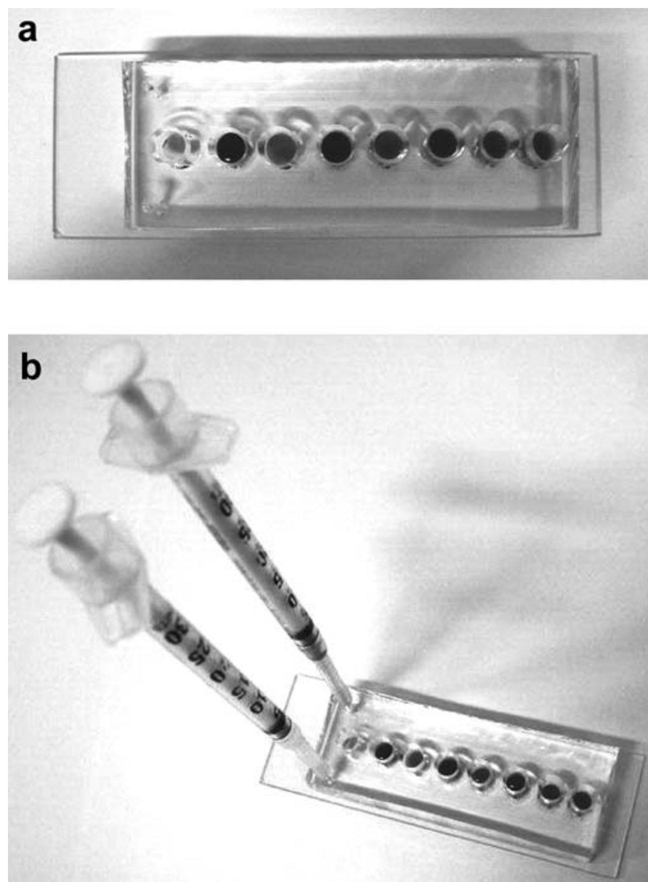
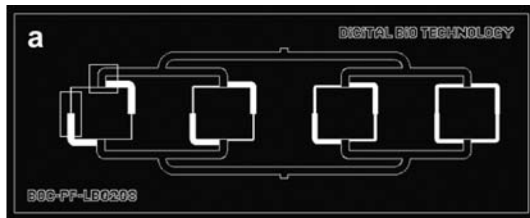
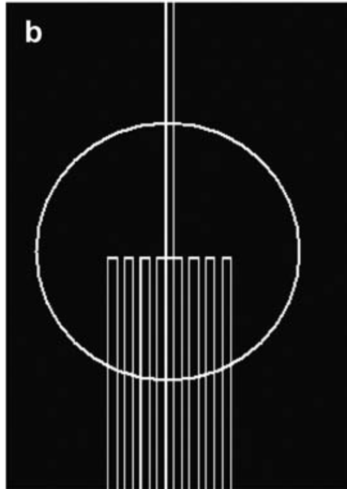



**Fig. 2.** **a** Fabricated PDMS microchip for serial dilution. **b** Sample and buffer solutions are injected manually into the microchip independently using two disposable plastic syringes



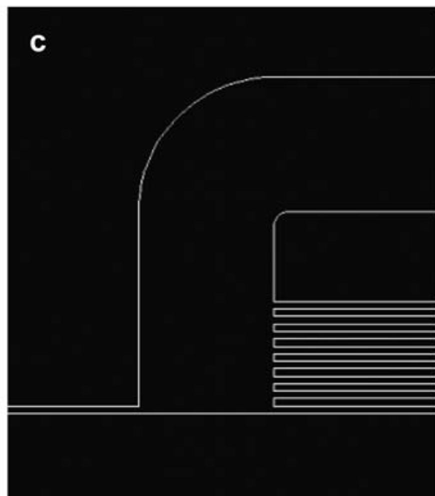




**Fig. 3.** **a** Channels initially split from two inlet ports are finally separated into 8 mixing chambers. **b** White circle indicates the position of the hole that will be punched later for the well, and through the lower 8 channels flow comes in 8 times more in quantity than the upper single one. **c** Distributing flow exactly with an integer-to-integer ratios by the numbers of the subchannels in the watershed is the main logic of the serial dilution microchip

Inspecting the accuracy while operating the microchip, 300 μl of the water-soluble dye solution (Tryphan blue) and de-ionized water were prepared and syringes containing each of them were connected to the sample and buffer inlet port respectively. By merely squeezing the syringes, diluting in 8 wells with linearly increasing concentration was completed. The concentration was inspected by spectrophotometer at 532 nm wavelength. Then, the chip was cleansed by pumping in 70% ethanol solution thorough both inlet ports and blowing up the resident ethanol solution with high-pressure air gun through inlet ports again so as for the chip to be reused. Data plotted in Fig. 4 were acquired by repeating this progress 5 times using the same chip. The measured data points have an average linear deviation of ±1%. This shows that a higher accuracy of serial dilution can be achieved in less than a minute.

**Table 1.** Sample concentrations listed above are the ratios of buffer to sample or sample to buffer; the order does not have any significance on the symmetry of the concentrations generated

| Port number ($n$-th well from left) | Number of the upper (sample) subchannels | Sample concentration | Number of the lower (buffer) subchannels |
|---|---|---|---|
| 1 | 1 | 1/9 | 8 |
| 3 | 2 | 2/9 | 7 |
| 5 | 3 | 3/9 | 6 |
| 7 | 4 | 4/9 | 5 |
| 8 | 5 | 5/9 | 4 |
| 6 | 6 | 6/9 | 3 |
| 4 | 7 | 7/9 | 2 |
| 2 | 8 | 8/9 | 1 |



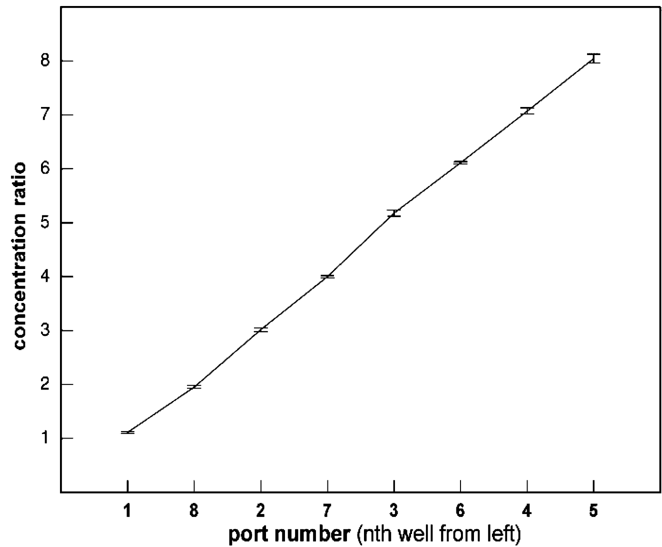



**Fig. 4.** Absorbance of light at 532 nm wavelength from each well measured with spectrophotometer

## 4 Conclusions

We made a plastic microchip which can provide an essential function required in the LOC (Lab-on-a-chip) industry using polymer microfabrication technology [12] and proved that it is much more accurate and efficient than the conventional macro-scale tools that are used nowadays. With the flexibility in channel design and efficiency in handling liquids, this kind of microchips can play important roles in future LOC industry. Especially in mixing, we adopted a macro-scale mixing method to avoid all the micromixing problems more efficiently and reliably. The method that applied while producing the disposable plastic serial dilution microchip, enables the design of

cheap and flexible miniaturized microfluidic channel devices.

In contrast to most alternative techniques, in which mixing induces time-dependent diffusion between flow layers, mixing in a macro-scale well chamber was robust and reliable. This kind of LOC is believed to replace the tedious manual work in connection to the serial dilution process in biology or chemistry laboratories that requires the carefully defined concentrations, which at the same time, guarantees the reproducibility and the reliability.



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