ON-DEMAND SERIAL DILUTION USING QUANTIZED NANO/PICOLITER-SCALE DROPLETS
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ABSTRACT
This paper describes a fully automated droplet-based microfluidic device for on-demand serial dilution that is capable of achieving a dilution ratio of >6000 (concentration range from 1 mM to 160nM) over 35 nanoliter-scale droplets. This serial diluter can be applied to high throughput and label-free kinetic assays by integrating with our previously developed on-demand droplet-based microfluidic with mass spectrometry detection.

KEYWORDS: Droplet microfluidics, Serial dilution, Concentration gradient, Mass spectrometry

INTRODUCTION
Serial dilution—stepwise dilution of reagent in a solution—is a standard practice for assays in enzyme and inhibitor kinetics, cytotoxicity, bacterial chemotaxis, and drug-discovery. Serial dilution is generally achieved by using pipettes, multiwell plates, and robotic platforms. Challenges associated with conventional serial dilution methods include large (microliter to milliliter) sample volumes, tedious manual sample handling, problems in conducting experiments with precious samples such as purified proteins, and compounding errors during each successive dilution. To address these challenges, in the past decade, many microfluidic dilution devices have been developed.1-3 These devices, however, often require very large footprints, a prime property in device design, to achieve serial dilution through network of channels.3-5 In addition, the programmability in these devices is difficult to achieve due to passive control of flow parameters1,3,6 and predesigned dilution ratios resulting from fixed fluidic channel dimensions.4,7,8

Figure 1: Comparison of major droplet-based dilution strategies | among above three strategies, the log-scale serial dilution could be achieved only through the present system.

EXPERIMENTAL
The PDMS device is fabricated using a single photomask9 designed according to multilayer soft lithography technique. We demonstrate a very compact and on-demand serial dilutor that generates quantized droplets and subsequent serial dilution by using a mechanical valve-based injection method10,11 (Figure 1c). The mechanical valve based injection method is highly flexible and independently controls the timing of droplet formation, the droplet size, merging and mixing. Our diluter consists of a chamber
(C), an oil channel (O) where the droplets are generated, and two ports, reagent port (R) and buffer port (B). To operate the diluter, the chamber is first filled with reagent by opening the appropriate valve (Figure 2a-b). The dilution is achieved by opening the buffer valve for small time to inject a small amount of buffer into the chamber. Due to conservation of volume in the chamber, when the buffer is injected into the chamber, an equivalent volume emerges from the chamber into the oil channel in the form of a droplet (Figure 2c-e). Therefore, the first and last droplets carry 100% reagent and 100% buffer, respectively. The remaining droplets, from first to last, contained decreasing reagent concentrations.

![Diagram of diluter operation](image)

Figure 2: Working principle of the our compact and on-demand serial diluter.

RESULTS AND DISCUSSION

To characterize our dilution method, we established the relationship between droplet number and corresponding concentration droplets and plotted a dilution profile. To plot dilution profile, we generated a series of 35 droplet microreactors containing different concentrations of the fluorescein, where its concentration was varied from 1 mM to 160nM (Figure 3). The fluorescein concentration inside each droplet was determined from the intensity of the initial fluorescein droplet observed in the captured video.

![Dilution profile graph](image)

Figure 3: Dilution obtained from present system. The results demonstrate dilution ratio of 6230 (concentration ranges from 1mM to 160nM), achieved in 35 quantized nanoliter-scale droplets.

CONCLUSION

In conclusion, we demonstrate very large scale serial dilution ratio of >6000 (concentration ranges from 1 mM to 160nM) that was achieved over 35 quantized nanoliter-scale droplets. We have also shown that the shape of the calibration curve can be tailored by altering the droplet generation frequency or the
shape of the mixing chamber. In combination with previously developed on-demand droplet merging and coupling with mass spectrometry, this serial dilution capability will enable high throughput, label free determination of enzyme kinetic parameters.

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