A PICOLITER DISPENSER WITH DISPOSABLE CARTRIDGES FOR PRECISE AND CONTACT-FREE INJECTION OF DNA INTO OPEN MICROFLUIDIC STRUCTURES

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ABSTRACT

We present a new disposable liquid handling system addressing the lower picoliter volume range and allowing for precise and contact-free injection of biopolymer samples into open microfluidic structures. Droplet placement precision of single stranded DNA (ssDNA) solutions up to 50 µM and a DNA reference ladder is investigated by high resolution optical monitoring. We present three different sample injection methods that account for evaporation protection of picoliter samples. Finally, we show the applicability of the dispenser by a successful electrophoretic separation *via* injecting ~70 pL of a DNA solution into a 200-300 µm wide gel line.

KEYWORDS

contact-free picoliter dispenser; open microfluidic gel electrophoresis; DNA injection.

INTRODUCTION

Open microfluidics (OM) has evolved as a promising technology in which fluidic structures are generated and accessed on planar substrates without rigid-rigid interfaces like micro-fabricated channels [1]. The concept allows for low-cost rapid prototyping of fluidic structures [2]. Recently, electrophoretic separation of DNA in direct-written open gel lines has been demonstrated [3]. The two key steps for reproducible separations in such structures are the generation of liquid separation channels and the precise contact-free sample injection. Here, resolution of electrophoretic separations is mainly determined by the injected sample plug length and hence is inversely related to its volume. Therefore, a dispenser capable of precisely injecting picoliter samples in a non-contact manner is highly desirable. Due to the open interface it is necessary to provide protection against evaporation of liquids which becomes more important the smaller the liquid volumes of the assay are. A successful strategy is to cool the substrate close to the dew point and to cover liquids with oil [3]. It is therefore crucial that the sample injection method is compatible with the evaporation protection.

Here, we present a non-contact drop-on-demand dispenser that is capable of reproducibly placing droplets down to 35 pL into open microfluidic gel lines. We propose a method to measure and quantify placement precision and investigate three different sample injection methods that account for the evaporation protection of the liquid gel line and the sample. Further, the presented dispenser features low-cost disposable cartridges, has no thermal impact on the sample, and can be applied to biopolymer and salt solutions. Here, we present data for ssDNA concentrations up to 50 µM and for a standard DNA ladder.

DISPENSER TECHNOLOGY

The dispenser chip is a silicon-glass compound comprising a dosage chamber with a backside membrane, a fluidic inlet, and a 20x20 µm² orifice (Fig. 1 a-b). In brief, the silicon-glass chips were fabricated on a wafer level as follows: After lithographic structuring of a photoresist, the fluidic chamber was 20 µm deep etched into the silicon by deep-reactive ion etching (DRIE). Through-holes for the fluidic inlet in the glass (Pyrex) were fabricated by a wet etching process. Both wafers were bonded anodically and subsequently diced. The disposable cartridge is finally assembled by gluing the silicon-glass chip to CNC-milled chip holders that comprise the medium reservoir (Fig.1 d). Both, chip and holder undergo a specific cleaning procedure to minimize organic contamination. To prevent wetting of the nozzle, the exterior of the chip is coated by a vapor phase deposited organo-silane after O_2-plasma treatment: A highly hydrophobic monolayer is formed on the silicon/glass surface preventing precipitation of solutes ensuring reliable and reproducible droplet generation of salt and polymer solutions. The cartridge is operated in a direct-displacement mode where a piezoelectric driver (BioFluidix P9) deflects the silicon membrane resulting in on-demand single droplet generation. Droplet volume is adjustable within a specific range, mainly depending on orifice dimensions, and the piezo stroke-length [4].

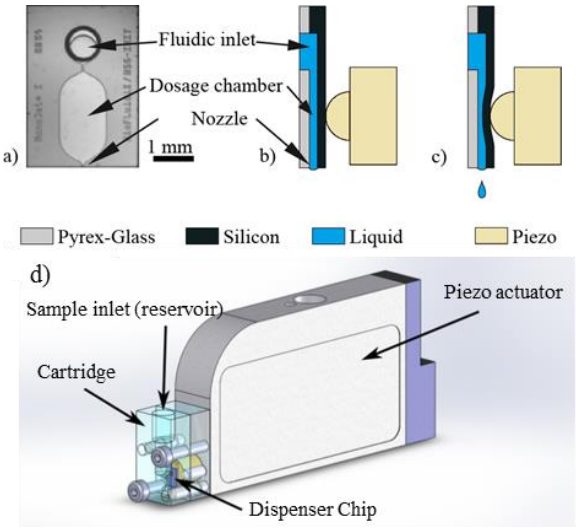

Figure 1: The dispenser chip (a) consists of a micro-fabricated silicon-glass compound (b). Droplets are generated by deflecting silicon membrane driven by a piezo actuator (c). The CAD model (d) illustrates the disposable cartridge comprising the dispenser chip assembled with the piezo actuator (BioFluidix P9).

Droplet volume

Droplet volume is measured by the gravimetric method introduced in [5]. In brief, we dispense into a weighing dish which is prefilled with silicone oil to prevent evaporation of the dispensed droplets. For each measurement point 600 droplets were dispensed at a frequency of 40 Hz. Thereby, we derive a highly accurate measure of the average droplet volume. Fig. 2 illustrates the characteristic dosage curve of our dispenser for water based solutions of 30 & 70 basepair (bp) ssDNA with equimolar concentrations ranging from 0.1 µM to 50 µM. Further, we used a DNA ladder (25-500 bp / 9 fragments; 576 nM all together in TE-buffer).

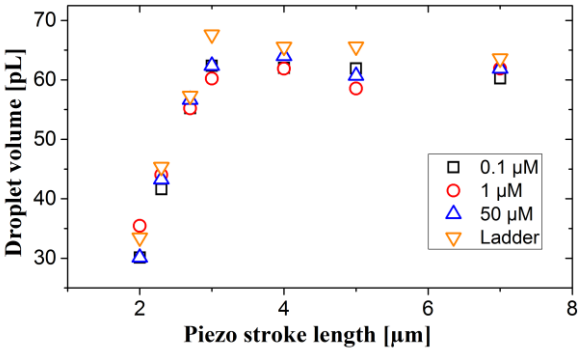

Figure 3: Dosage curves of the dispenser chip with ssDNA concentrations ranging from 100 nM to 50 µM (30 & 70 bp, equimolar) and a standard DNA ladder (576 nM). 600 droplets were dispensed for each data point and measured with the gravimetric setup. The droplet volume can be tuned between 30-60 pL.

Varying the piezo stroke-length between 2-3 µm volumes ranging from 30 to 60 pL are obtained. At higher stroke lengths a characteristic plateau in the droplet volumes is observed. In this regime we frequently observe formation of satellite droplets. The volumes vary between the different DNA samples by 9 % CV for ~ 35 pL droplets and 2 % CV for ~ 50 pL.

Droplet stability & placement precision

In order to investigate reproducibility and placement precision, droplets are dispensed on a glass slide (working distance: 2.5 mm) which is continuously monitored by a camera from below with a lateral resolution of approximately 2 µm. The glass slide was rendered hydrophobic with windshield treatment (rain-x original glass treatment) which improves the contrast of the images. For each sample we determined the center of 100 subsequently dispensed droplets with Matlab using a custom image processing script. The setup and workflow is illustrated in Fig. 4. In order to quantify placement precision of the dispenser we calculate the deviation of two consecutively dispensed droplets which we refer to as drop-to-drop deviation d_{mean} (averaged over 100 droplets) and d_{max} (maximum out of 100 droplets). Further, we define the scattering radius (eq. 1)

$$R_{2\sigma} = 2 \bullet d_{std,} \qquad (1)$$

where d_{std} is the standard deviation of the droplet position and therefore $R_{2\sigma}$ reflects the size of the circle in which 95 % of the dispensed droplets are located considering a normal distribution. In Fig. 4 c) $R_{2\sigma}$ is illustrated as a red circle. It ranges from 4 µm (DNA ladder) to 7 µm (50 µm ssDNA). The maximum drop-to-drop deviation d_{max} for DNA ladder is below the setup's detection limit and was highest for 50 µM ssDNA (5.9 µm). The resulting values are summarized in Table 1.

Table 1: Scattering radius $R_{2\sigma}$, mean and maximum drop-to-drop deviation (d_{mean}& d_{max}) for 100 subsequently dispensed droplets of the tested samples. All values are given in µm. Entries marked with an asterisk are below the detection limit.

Sample	$R_{2\sigma}$	d_{mean}	d_{max}
100 nM ssDNA	4.8	2.7	4.3
1 µM ssDNA	5.1	2.8	5.5
50 µM ssDNA	7.0	2.3	5.9
DNA ladder	4.0	*	*

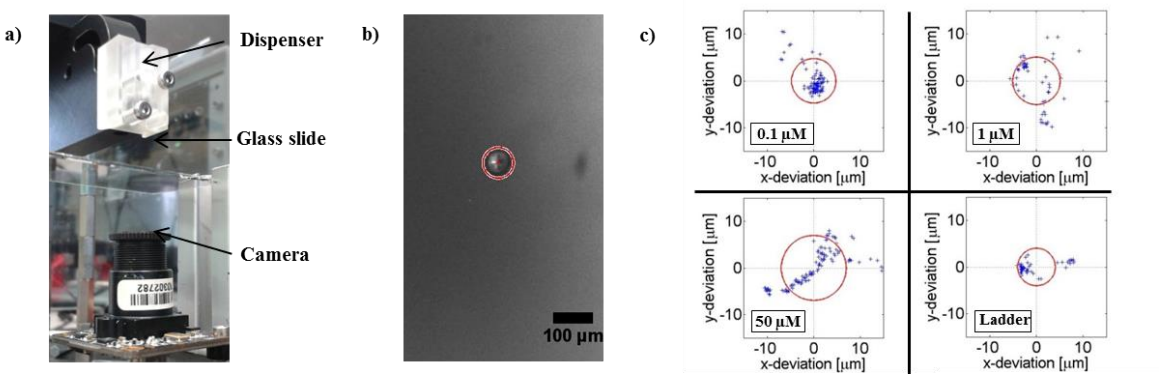

Figure 4: Measurement setup and workflow to determine droplet placement precision. Droplets are dispensed on a glass slide and imaged by a camera from below (a). The center of each 60 µm wide droplet is determined with a Matlab script (b). The positions of 100 subsequently dispensed droplets are shown in the scatterplots (c). The red circle illustrates the scattering radius $R_{2\sigma}$. Note, that in all cases scattering radius is significantly smaller than the size of the droplet on the substrate.

Sample injection in open microfluidics

To perform electrophoretic separations in open microfluidics key features are the generation of a liquid separation channel across two electrodes on a planar substrate, the contact-free sample injection, and evaporation protection realized by a covering oil layer and cooling. Here, the separation channel is made by direct writing of a linear polyacrylamide hydrogel [3]. In order to inject DNA into open microfluidic structures the presented dispenser was mounted to the 3-axis robot that is responsible for structure generation. By the use of a non-contact dispenser as injector the injection plug length is defined by the droplet volume and is an indicator for resolution. Thus, small volumes are beneficial for a good separation performance as long as detector signals are significant. During impact of droplets on the channel liquid their transferred kinetic energy results in liquid spilled around and injection length is larger compared to the initial droplet diameter. Hence, volume and kinetic energy of injected droplets should be small. Using the presented dispenser three injection methods have been identified to inject small amounts of only 35 pL ($d_{droplet} \approx 41$ µm) into 200-300 µm wide gel lines. Fig. 5 shows the fundamental process of open microfluidic system prototyping (left) and the three different injection methods investigated in this study (right). To illustrate the applicability of the dispenser we injected single ~35 pL droplets of fluorescently labeled (Atto 532) ssDNA sample (30 & 70 bp, 50 µM) exhibiting constant injection lengths of only ~150 µm as shown in Fig. 5 a-c.

Spatial drop-to-drop deviation of DNA was compensated by a referencing method before each injection, whereby all droplets were placed sufficiently close to the center of the line. In Fig. 5 a) DNA was directly dispensed into the gel after line generation, followed by oil coating. In Fig. 5 b) a rehydrated injection spot can be seen. Here, the DNA sample was first dispensed onto the blank substrate and then dried. Next, the gel line was written over the dried spot which leads to rehydration of the DNA, followed by coating with oil. Finally, in Fig. 5 c) the dispenser was used to directly inject the DNA sample into the oil coated line. Here, we added 0.1 % (v/v) of a surfactant (Span 80) to the silicone oil (200 Fluid, Dow Corning) in order to decrease surface tension which allows for rapid penetration of the dispensed sample droplet into the oil layer. Due to the fact that in most cases open microfluidic systems are non-equilibrated immediately after line generation, the injected DNA spot sometimes smears out when applying injection method a). Longer idle-times before injection and oil coating are disadvantageous due to evaporation. Method b) is reproducible but only applicable for biomolecules that are stable during drying and that can be rehydrated like DNA. Method c) is applicable for all biomolecule solutions but reproducibility must be evaluated in the future. In Fig. 5 d) the live separation of the aforementioned DNA mixture is demonstrated using injection method c) applying an electric field of 50 V/cm. Due to the low sensitive live detection setup comprising a green DPSS laser (λ_{ex} = 532 nm, 4 mW, Blau Optoelektronik GmbH) with line optics and a CMOS camera with a pre-mounted long-pass filter (λ_{cutoff} = 550 nm) 70 pL were injected for a better visualization of the live migration. First separation is achieved 90 s after applying the electric field at a migration distance of ~3 mm.

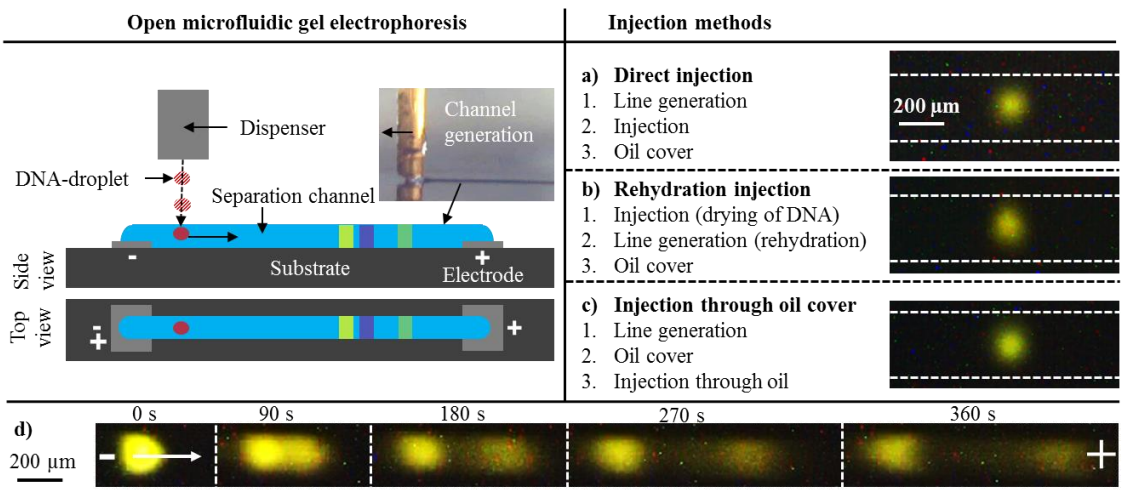

Figure 5: System generation for electrophoretic separation of fluorescently labeled DNA (Atto 532). The gel line acting as liquid separation channel was written in a semi-contact method across two platinum electrodes on a planar Kapton 500B substrate. DNA is contact-free injected using the presented dispenser. To prevent evaporation substrate is cooled & covered with oil. In a), b) & c) different injection methods are presented visualized by a live detection setup (exposure times: 500 ms). d) Separation process of ssDNA-fragments (30- & 70-bp; 50 µM) at 50 V/cm. Pictures were taken at different timestamps.

CONCLUSION

We presented a new dispenser for the lower picoliter range that can be used to precisely deliver biopolymer solutions contact-free without any thermal impact on the sample. We proposed a new method to evaluate placement precision by high resolution optical monitoring. Droplet volume and precision for various DNA samples have been evaluated. Three different methods have been identified and investigated that were successfully used for the injection of DNA samples in open microfluidic structures. In practice the injection through a previously placed oil cover might prove to be the most universal and reliable method, as it is likely to be compatible with other samples like proteins and limits the time the open gel line is exposed to air. Even quite high concentrated biopolymer solutions (50 µM DNA, 2x25 µM respectively) could be reproducibly injected into OM structures exhibiting injection lengths as small as ~150 µm followed by successful electrophoretic separation. Given the high placement precision of the dispenser we can operate from a fairly large working distance of 2.5 mm. All 35 pL droplets (diameter ~41 µm) were sufficiently placed in the center of 200-300 µm wide gel lines. The high dispensing precision allows for controlled DNA injection into even narrower structures, whereby separation performance could be improved. Summed up, the presented dispenser is capable to reproducibly dispense droplets with volumes down to 30 pL exhibiting exceptionally high placement precisions from large working distances with even high DNA concentrations.

In future we will further exploit the methodology of placement precision measurement and the range of applicable samples. Since a calibration-free dispenser could be beneficial for many applications, the variation within different disposable cartridges needs to be fully characterized as proposed in [6]. From our point of view, precise contact-free injection of picoliter samples combined with protective oil covers are not only of interest for OM electrophoresis but also for other biochemical assays such as solid phase DNA amplification or single cell studies in oil protected droplet arrays.

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