TopSpot Vario: New Method for Parallel Nanoliter Dosing by Direct Liquid Displacement

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Abstract

TopSpot® printheads are used for the highly parallel nanoliter dispensing of bio-chemical substances at a pitch of typically 500 µm [1,2] . This paper reports on a new and improved way of operating the printheads, using an incompressible material between the piezo actuator and the dispensing medium [3,4]. The advantage of an incompressible medium is the direct relation between the amplitude of the piezo-stack actuation and the ejected liquid volume. Earlier reports stated that the filling of the printheads is a key issue. In this paper we report on the implementation of an already reported microchannel design for bubble free priming of blind channels [5] into the printhead design. The evaluation of the 24 channel TopSpot Vario printheads revealed that droplet volume can be freely tuned between 250 pl and 1600 pl for aqueous solutions. Best printing performance with an inter and intra nozzle reproducibility of droplet volumes of 7.5 % respectively 1.9 % was found for droplet volumes of 270 pl. Stable printing could be provided up to printing frequencies of 400 Hz and for liquids with viscosities up to 11 mPa·s.

1 Introduction

For the high throughput production of microarrays a highly parallel method for dispensing nanoliter droplets is needed. We previously reported on highly integrated TopSpot printheads with 24 and 96 channels, operated by compression of air [1,2]. In this paper we report on the use of a new actuation technology (TopSpot Vario) relying on an incompressible medium. This guarantees a cross contamination free filling of the printhead and a full control of speed and size of the ejected droplets [3,4]. Our choice of incompressible medium is an elastomer material, Sylgard 186 from Dow Corning. The TopSpot Vario print module consists of a silicon micromachined printhead and a piston (**Fig. 1**). The piston is actuated by a piezo stack actuator. Between the piston and the printhead the elastomer is placed. The movement of the piston is transferred through this, to the liquid to be ejected.

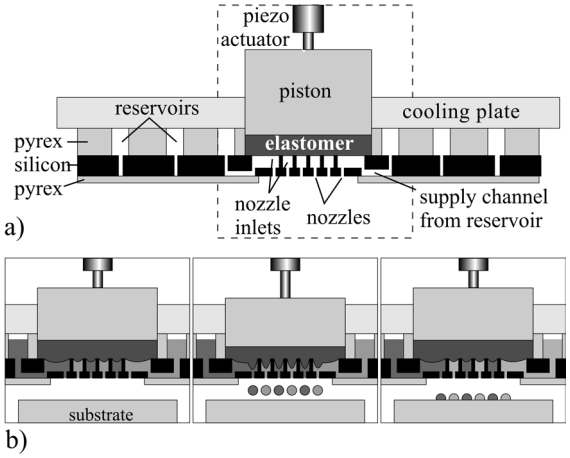


Figure 1. Schematic view of the TopSpot Vario set-up (top) and working principle (bottom)

The filling of the printhead takes place through capillary forces. Surface tension transports the liquid from reservoir to ejection nozzle. Also the refilling after droplet ejection takes place through capillary forces. The key problem during the filling of the TopSpot Vario printheads are air bubbles that can be trapped inside the nozzle inlets which are sealed by the elastomer. This prevents the actuation energy to be fully coupled into the liquid ejection.

In earlier experiments [3] it was not possible to reliably prevent cross-contamination between the channels when placing the elastomer after the printhead has been filled with the bio-chemicals. It is therefore absolutely necessary to place the rubber and seal the microchannels before the printhead is filled. In the following we describe a new layout of the microchannels for a reliable self-filling performance of TopSpot Vario printheads.

2 Printhead design

Trapping of air inside the channel system can be avoided by a two step channel structure as depicted in **Fig. 2**. In **Fig. 2a** the fundamental principle is illustrated and

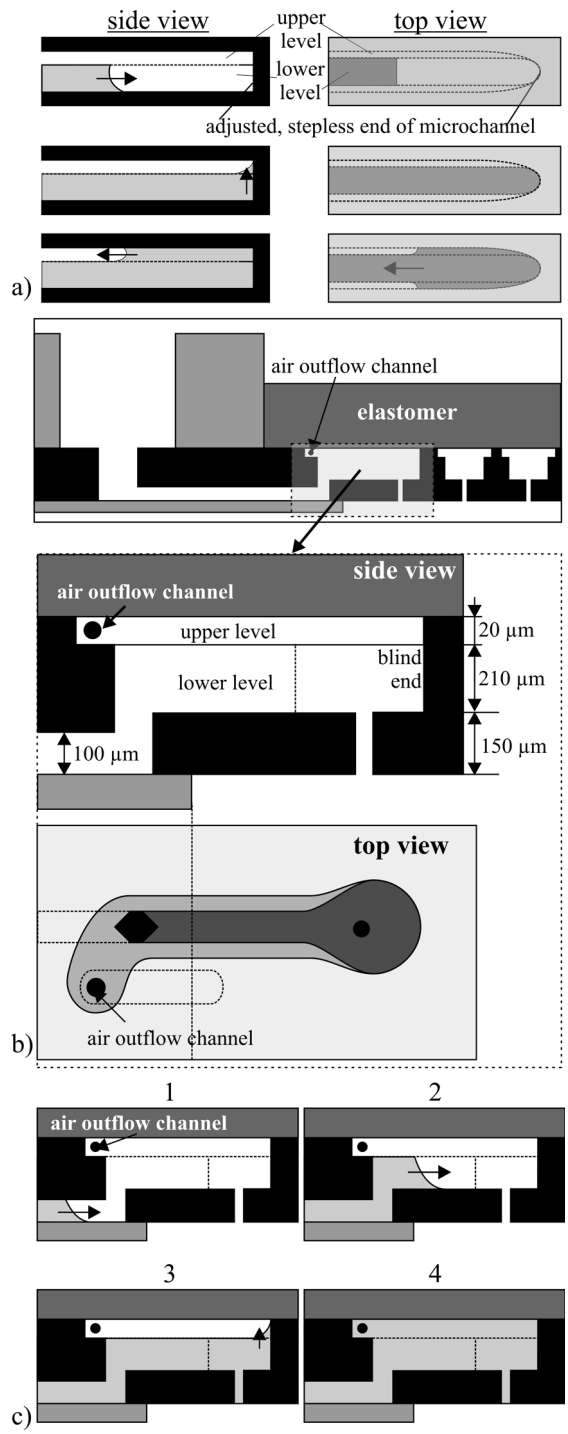
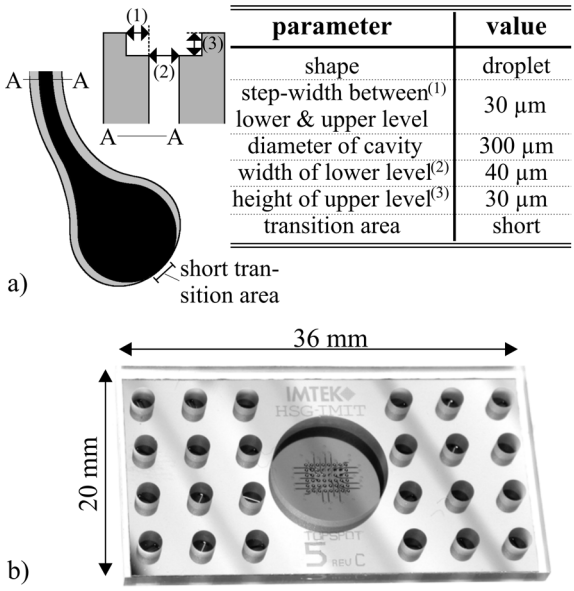


Figure 2. The new 2-level microstructure for prevention of trapped air bubbles in blind channels **a)** fundamental principle [5]: liquid is fed into the lower level (top). A sharp step between lower and upper level prevents liquid transfer into the upper level. At the blind end the sharp step is removed and changeover is enabled (middle). After liquid transfer the upper level is filled in reverse direction displacing air out of the channel inlet (bottom) **b)** implementation of the principle into TopSpot printhead design and typical geometries **c)** working principle of bubble free priming of TopSpot nozzle inlets and corresponding inlet channels

Fig. 2b shows the implementation of the principle into the TopSpot Vario printhead design. The working principle is explained by the cross sections in **Fig. 2c**. Liquid flows from the microchannels on the bottom side of the silicon layer to the via. There it changes to the top side into the lower level of the nozzle inlet channel and flows towards the nozzle funnel. In the nozzle funnel the liquid reaches the top level of the channel to run back towards the air outflow channel. A capillary barrier preventd the liquid from running any further. As last preparation step the prestress on the elastomer is raised to seal off the air outflow channels. Extensive tests were made in order to identify the optimum layout for bubble free priming of the sealed microchannels [5]. The resulting suggested design rules for an optimized design are listed in **Fig. 3a** and a fabricated TopSpot Vario printhead with implemented optimized design is shown in **Fig. 3b**.



parameter	value
shape	droplet
step-width between(1) lower & upper level	30 µm
diameter of cavity	300 µm
width of lower level(2)	40 µm
height of upper level(3)	30 µm
transition area	short

Figure 3. Optimized printhead design **a)** suggestion rules for 2-level TopSpot Vario nozzle inlets and nozzle inlet channels [5] **b)** TopSpot Vario printhead with implemented 2-level microfluidic structures

3 Experiments and Results

The optimized printhead design was tested for its printing behaviour with respect to the piezo actuation and the prestress on the elastomer. As printing behaviour the ejected volume and the speed of the ejected droplets were measured together with their coefficient of variation (CV). These were measured from the images taken with a stroboscopic camera (Mocon-RT). Time calibration was taken directly from the camera timing. The geometrical calibration was done from the distance between two ejection nozzles (500µm).

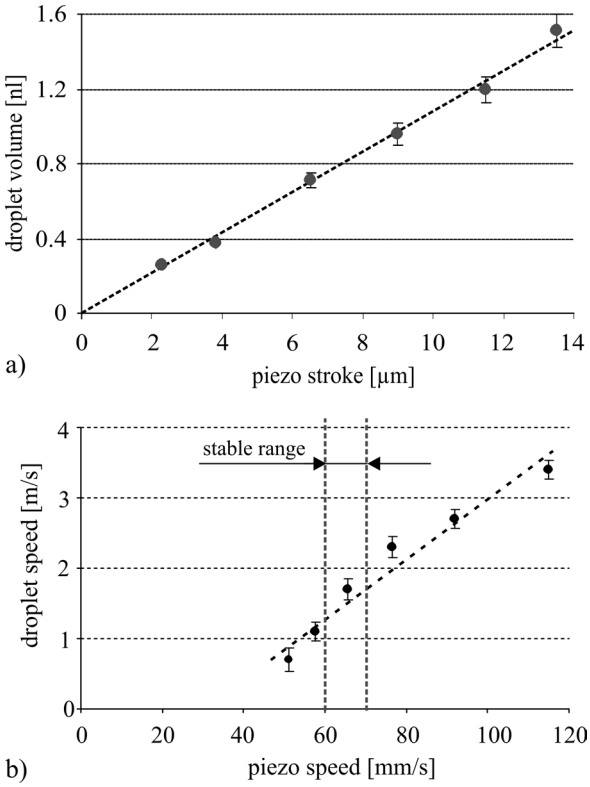
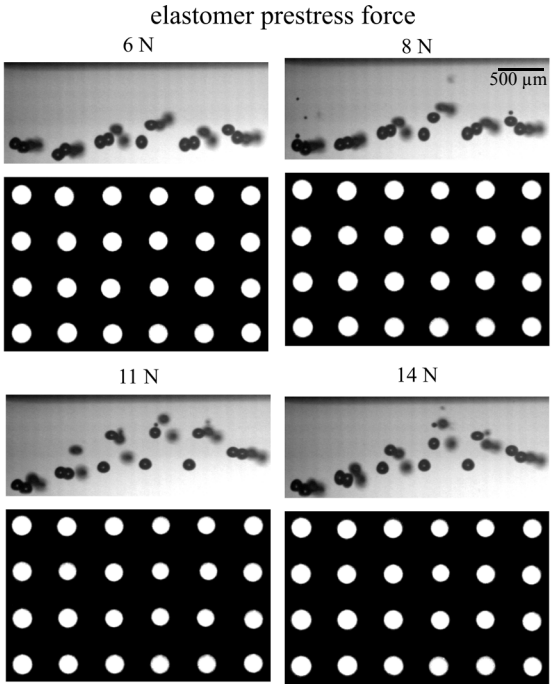


Figure 4. Influence of piezo movement on droplet volume and speed **a)** droplet volume versus piezo stroke. Error bars represent the CV of 5 % of mean droplet volume of all 24 droplets of one array between the experiments **b)** droplet speed versus piezo speed. Error bars represent CV of droplet speeds of all 24 droplets in one array.

As expected, the ejected droplet volume and speed are correlated directly to the stroke and speed of the piezo actuator (**Fig. 4**). An increasing CV of all 24 droplet volumes in one array (inter nozzle reproducibility) was observed with increasing piezo stroke. The inter nozzle reproducibility of droplet volume increased from 7.5 % at a small piezo stroke (2.5 µm) up to 10.2 % at maximum piezo stroke (13.5 µm).

Concerning piezo speed it was already reported, that there exists a certain stable range where exactly one droplet is ejected out of each nozzle [3]. Speeds above that range produce satellite droplets following the main droplet and speeds below result in an unstable droplet tear-off. This observation could be verified again. The dotted lines in **Fig. 4b** give the stable range which was found for piezo speeds between 60 mm/s and 70 mm/s at a piezo stroke of 5 µm.

The prestress force on the elastomer did not influence the absolute values of the speed and volume remarkable in the tested range but it influenced the CVs of these.



prestress force [N]	6	8	11	14
droplet volume [nl]	1.6	1.5	1.6	1.5
spot diameter [µm]	270	250	250	250
CV of spot diameters [%]	3.6	4.2	5.3	5.6
CV of droplet speeds [%]	11	14	26	29

Figure 5. Influence of elastomer prestress force on droplet and spot homogeneity respectively. Top pictures show camera records of ejected droplet arrays and bottom pictures show resulting microarrays recorded by the fluorescence scanner. The table lists mean droplet volume and spot diameter of all droplets/spots in one array as well as the CVs of droplet speed and spot diameters of all droplets/spots of one array.

The variation in droplet volume was measured for various prestress forces (**Fig. 5**). For these experiments the droplet volume was not only measured from the stroboscopic images but also through the reading of a fluorescence scanner (Bioscanner, LaVision Biotech). The diameter was taken from each spot, as measure for the diameter of the corresponding ejected droplet. The records as well as the listed values in **Fig. 5** show that with increasing elastomer prestress force the droplet homogeneity decreased.

To get the overall performance of the system, an experiment with small elastomer prestress force (6 N) and small piezo stroke (2.5 µm) was repeated ten times using a fluorescence marked aqueous solution (3M betaine/6xSSC + 2µM Dyomics DY555) as printing solution. An mean droplet volume of all 24 droplets of one array was measured at 270 pl with a CV of 5 % over all ten experiments. The inter nozzle reproducibility of spot diameters was in average 2.4 % and below 3.4 % in all experiments. The inter nozzle reproducibil-

ity of droplet volumes was measured at 7.5 % The intra nozzle reproducibility of droplet volumes (CV of volumes of 100 subsequently ejected droplets out of the same nozzle) was measured at 1.9 %.

Printing frequency was increased in 100 Hz steps to estimate the maximum frequency where stable operation could still be guaranteed. At a frequency of 500 Hz the first satellite droplet appeared and printing got unstable. This resulted in a maximum printing frequency of 400 Hz.

Additional experiments using different mixtures of aqueous glycerol solutions as printing medium were performed to estimate the maximum dispensable liquid viscosity. While 60 wt-% aqueous glycerol solution (11mPa s) could still be ejected in stable operation, no droplet tear off could be observed anymore using a 70 wt-% aqueous glycerol solution (23 mPa s). Furthermore, at constant piezo stroke, a decrease of droplet volume with increasing liquid viscosity was observed. At maximum piezo stroke of 13 µm the droplet volume decreased from 1600pl dispensed water (1 mPa·s) down to 600 pl using 60 wt-% aqueous glycerol solution (11 mPa·s) (see **Fig. 6**).

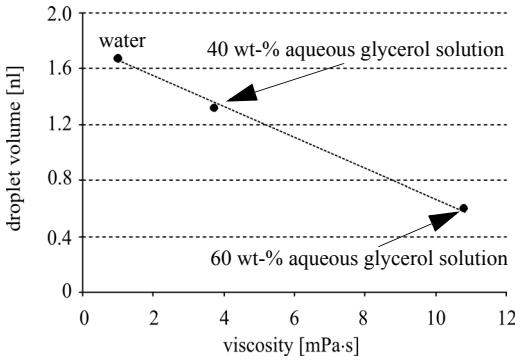


Figure 6. Droplet volume versus liquid viscosity at a constant piezo stroke of 13 µm

4 Conclusion and Discussion

We presented a novel method for parallel nanoliter dispensing which can be used for non-contact printing of microarrays. A fundamental principle for bubble-free priming of blind channels [5] was successfully implemented into the printhead design. This solved one of the major problems of the TopSpot Vario technology [3,4].

By adjusting the piezo actuator stroke the system allows to freely tune droplet volume in a range between 250 pl and 1600 pl for aqueous solutions. Stable operation, which means that only one droplet is ejected out of each nozzle, can be adjusted by tuning the piezo actuator speed.

The measured increase of inter nozzle reproducibility with increasing droplet volume suggests that higher spot diameters should be achieved by multiple printing of small droplets into each other instead of ejecting only one large droplet. Best printing performance was found at a droplet volume of 270 pl with an inter- and intra nozzle reproducibility of droplet volume of 7.5 % and 1.9 % respectively. The CV of droplet volume between different experiments was measured at 5 %. Printing was stable up to frequencies of 400 Hz and liquids with viscosities of up to 11 mPa·s (corresponds to 60 wt-% aqueous glycerol solution) could be successfully dispensed in stable operation.

With these results the TopSpot Vario system can compete with other state of the art microarrayers [6,7] and be used for highly flexible microarray production.

5 Literature

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