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# A tuneable and highly-parallel picolitre-dispenser based on direct liquid displacement☆

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**Abstract**

We present a new method for the highly-parallel and simultaneous delivery of a multitude of reagents in the picolitre range. This method is based on direct displacement of the liquids using an elastomer-stamp, which simultaneously actuates up to 96 dosing channels, at a pitch of 500 μm. We were able to tune droplet volume from 100 to 700 pl and droplet speed from 0.3 to 4 m/s using printheads with 50 μm diameter nozzles. In contrast to all other inkjet techniques the new direct displacement method enables the precise control of dispensing quantity in the picolitre range regardless of reagent viscosity.


*Keywords:* Highly-parallel; Picolitre-dispenser; Bio-molecule; Microfluidics

## 1. Introduction

Microarrays are a certain type of biochips that enable fast and highly-parallel analysis of bio-molecules. A microarray consists of many small spots (typically 200 μm in diameter), in which each spot is sensitive towards one particular bio-molecule. After reaction with an unknown mixture of substances each spot is analysed to see if it reacted. In this way the composition of the unknown mixture can be deduced. It is expected that microarrays will be the main analysis and diagnosis tool for the coming decades. Crucial for high throughput production of microarrays is a fast method for dispensing 100–10,000 different reagents in picoliter droplets, at a pitch of 200–500 μm to form the sensitive spots. In the past we presented a printhead with 96 channels at a pitch of 500 μm, firing simultaneously by pneumatic actuation [1,2]. Based on this TopSpot-technology a fully automated production line with an up to now unmatched throughput was built (Fig. 1).

The TopSpot-technology is based on a silicon micromachined (mainly DRIE) printhead (Figs. 1 and 2) sandwiched between two glass layers. A micromachined printhead is necessary for maximum accuracy. The spotting solution is filled in the reservoirs. Capillary forces will draw the liquid to the nozzles in the centre print window. A piston is placed in the print window defining an air chamber. When actuated by the piezo actuator the piston will compress the air in the chamber causing a pressure pulse, which is transferred into the liquid. This will eject the droplets.

It was seen that with the pneumatic actuation, reagents having different viscosities are dispensed with different volumes. A controlled adaptation of droplet volume and droplet speed is also limited. In this paper we present a new actuation method based on direct liquid displacement that overcomes these limitations.

## 2. Experimental

In the previous concept the pneumatic pressure was generated by a fast mechanical displacement of a piston, reducing the volume of the actuation chamber. A large stroke from the piston is needed to achieve a high enough pressure due to the compressibility of the air. This large stroke minimised the possibilities of controlling the exact pressure pulse. In the new, direct displacement, method the compressible air is replaced by an incompressible but deformable medium (e.g. rubber [3], Fig. 2). The movement of the piston forces the elastomer into the nozzle array, displacing the reagents (Fig. 3).

The aim is a parallel, homogenous and stable operation of up to 96 nozzles without cross-contamination. Stable

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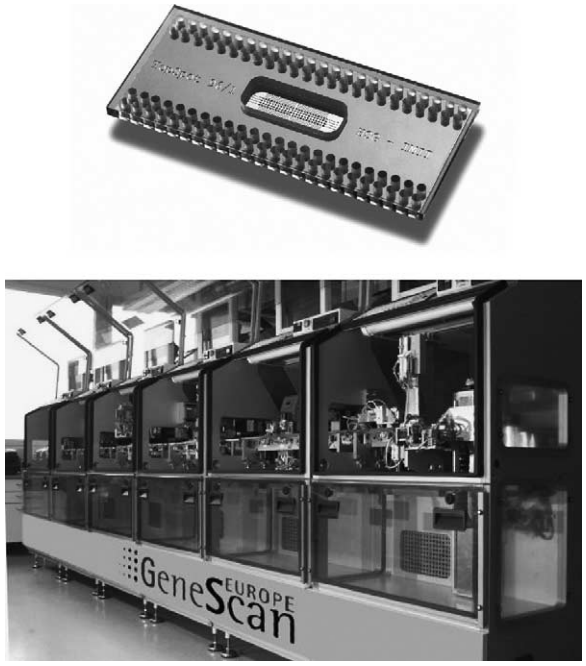


Fig. 1. Production line based on TopSpot-technology, spotting up to 1.440 different reagents at a throughput of 300 biochips/h, operated at Genescan Europe AG, Freiburg (D). Top: the 96 channel printhead.

operation means that exactly one droplet is ejected out of each nozzle without any following satellite droplets. A homogenous operation generates droplets, out of every printhead nozzle, with the same volume and speed.

The amplitude and speed of the piston movement is controlled at nanometer accuracy by a piezo-stack actuator. This piezo movement is transferred directly into the liquid due to the incompressibility of the rubber. That means that the piezo-stack actuation controls the movement of the liquid and so the dynamics of droplet ejection. Droplet volume can be tuned by actuator amplitude and droplet speed is adjusted by the piezo-stack velocity. The provided energy is limited to a specific range. It has to be high enough to overcome surface energy of the liquid in the nozzles, for

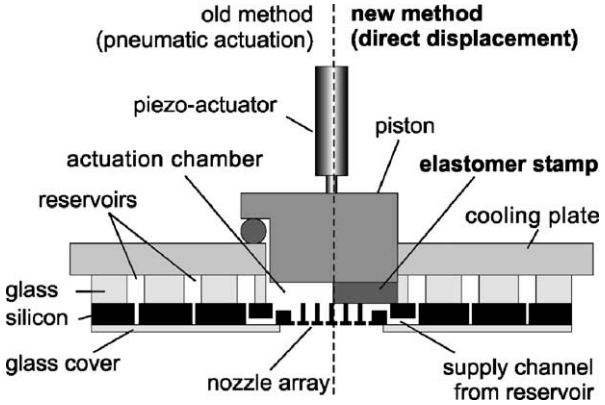


Fig. 2. Schematic of the old method based on air compression (left) and the new method based on direct displacement (right).

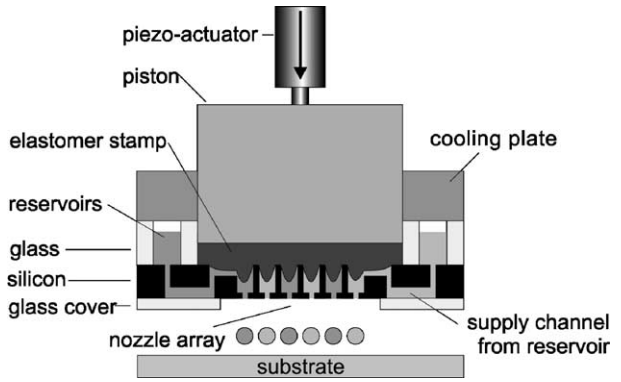


Fig. 3. Schematic of the elastomer deformation into printhead channels displacing the reagents.

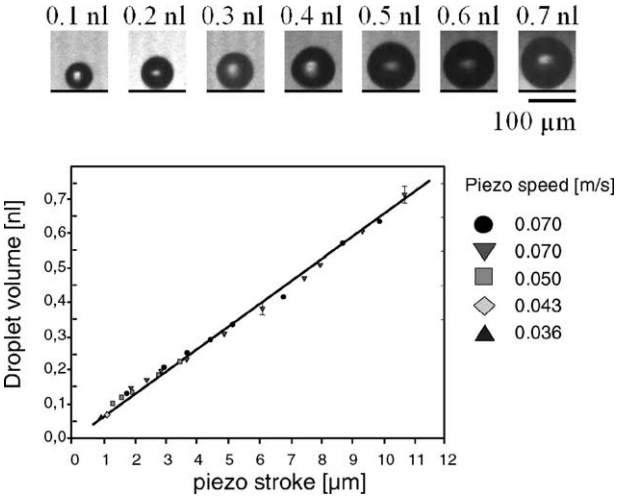


Fig. 4. The ejected droplet volume (nl) vs. piezo stroke (µm).

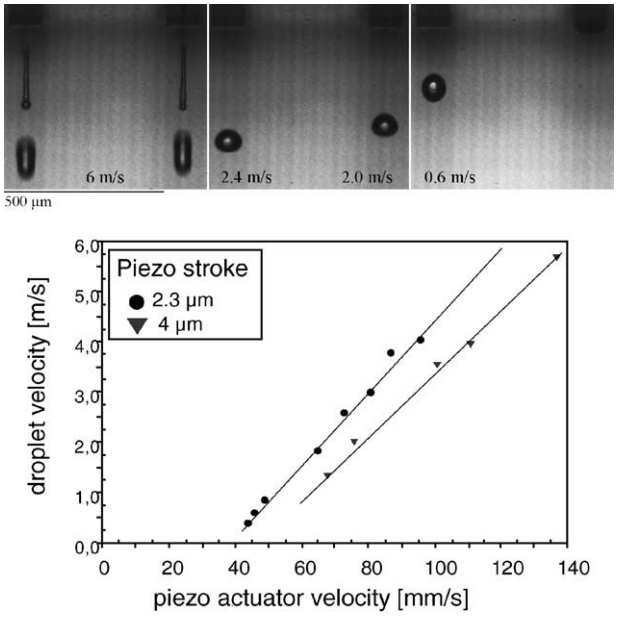


Fig. 5. Influence of the piezo speed ($10^{-3}$ m/s) on the droplet speed (m/s).

clear droplet tear off. The energy is limited to a maximum value, above which satellite droplets will appear [4].

First objective was to find the relation between actuator dynamics (stroke and speed) and the ejected droplets (volume and speed). In these experiments we focused on finding the parameter window for which stable droplet ejection is possible. The droplet ejection of one specific nozzle was recorded with a stroboscopic camera for measuring droplet volume and speed. Parallel droplet ejections of six nozzles in one row were recorded for investigations of the droplet homogeneity. Two different nozzle array designs were tested and the results compared.

Cross-contamination of the liquids in the nozzles has to be avoided during elastomer insertion into the printhead as well as during the printing process. Different elastomer-stamp geometries can be used to minimise the risk of cross-contamination during stamp insertion. We investigated different stamp shapes (Fig. 4) that guarantee a parallel approach between the elastomer and printhead surface. The incompressibility of the elastomer as well as the high stiffness of the actuator eliminates any feedback from liquid viscosity on the actuator stroke. This prevents cross-contamination during the printing process.

## 3. Results and discussion

The elastomer behaves like an incompressible medium and flows like a liquid for small deformations. For better understanding of the functioning of the direct displacement principle one could thus think of a direct fluidic contact between the actual piston and the ejection nozzles.

The ejected droplet volume as function of the piston stroke was measured (Fig. 4). A clear linear relation can

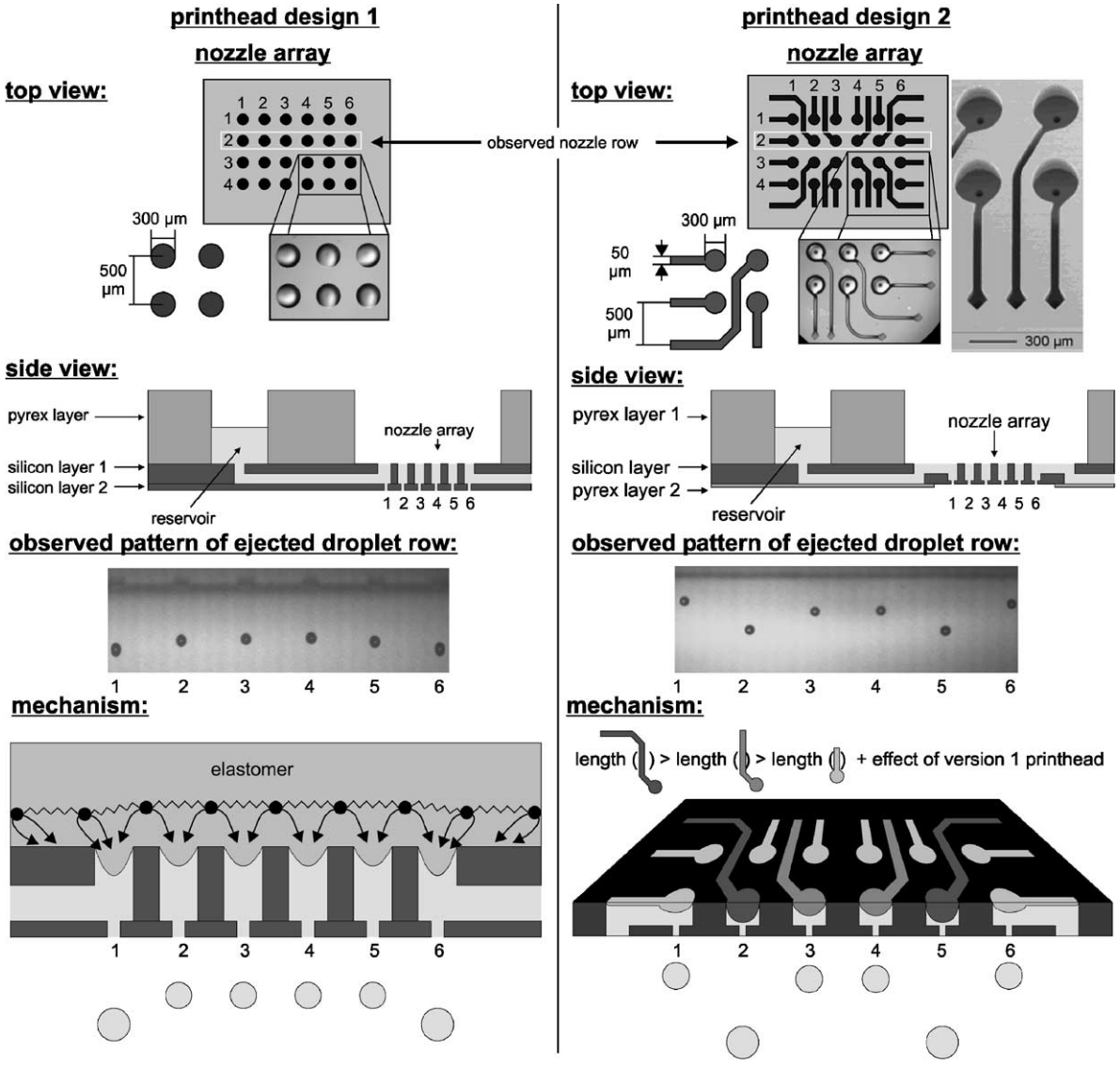


Fig. 6. The two investigated nozzle array designs that were investigated towards droplet homogeneity dependent of nozzle array type two different patterns of ejected droplets were observed.

be seen. The slope of the curve is 1, e.g. doubling the piezo stroke doubles the ejected volume. Important to notice is the zero crossing of the interpolation line and the very low deviation of the measurement points from this line. Secondly, it can be seen that the result is reproducible and there is no influence from the piezo speed on the droplet volume.

From the direct coupling of the piston and the droplet ejection it is also expected that the ejection speed is directly proportional to the piston speed. Several experiments were performed, where a certain piezo stroke was made in different time periods. Some results are shown in Fig. 5. There is again the linear relationship between the stroke speed and the droplet speed. The slope of the curve is 2, e.g. doubling the piezo speed quadruples the ejection speed. Secondly it can be seen that larger droplets (larger stroke) tend to a lower ejection speed. This can be explained by the energy that is used to build the droplets outer surface and the force it takes for the droplet to break clear of the nozzle. From the speed measurements it became clear that for each droplet volume there is a certain speed range for which the ejection is stable. In this typical smaller droplets (150 pl, Fig. 5) are stably ejected in a range from 0.4 to 4.0 m/s. The larger one (250 pl) is stable between 1.4 and 5.6 m/s.

Two different nozzle array designs were investigated towards droplet ejection homogeneity. First one consists of a six by four array of homogenous, round, nozzle openings. Second one has additional nozzle incoming lines with open tops (Fig. 6). The second row of six nozzles was filled for both printhead designs and their parallel droplet generation was recorded with a stroboscope camera.

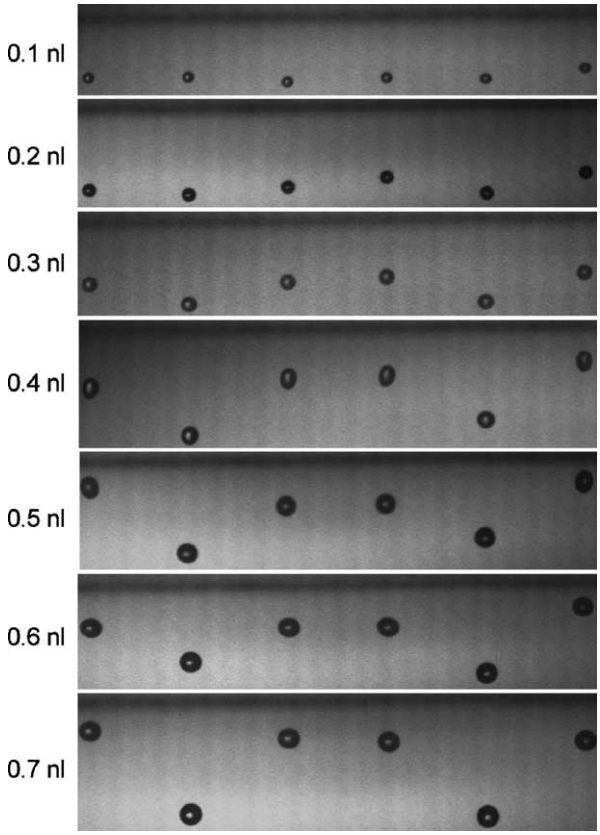


Fig. 7. Observed pattern of an ejected droplet row.

Printheads with a nozzle array of the first type eject a droplet row, where the first and sixth droplet are faster than the four others (Fig. 6, design 1). Volume deviation were close to the measurement error. The behaviour can be explained by an additional force component in the rubber occurring at the outer nozzles, which causes a higher elastomer displacement in the same time. From the previous two experiments it is expected that the effect on the ejection speed is much stronger then the effect on the ejection volume. This can also be seen in the observation photographs.

Nozzle arrays of second nozzle array type showed a droplet row where the second and fifth droplet are the fastest ones (Fig. 7). The different length of the nozzle incoming lines must be an addition factor here. Nozzles with long incoming lines have a higher contact surface between liquid and elastomer, which makes it easier for the elastomer to enter. This results in a higher displacement impulse during actuation. That would produce a pattern with fastest droplet out of nozzles 2 and 5 followed by droplets of nozzles 3 and 4. Due to the shortest incoming lines, nozzles 1 and 6 should generate the slowest droplets. The additional force compound, as seen in the previous experiment, at the outer nozzles has to be taken into account as well, which leads to the approximate same droplet velocity of droplets 1, 3, 4 and 6.

The corresponding influence on droplet volume was also verified here. Again the volume variation was below 5%, and the pattern was as expected (Fig. 7). The observed patterns can be eliminated by design changes of the nozzle array. Dummy nozzles and adjustments of channel length will generate a more homogenous droplet ejection. Despite the observed pattern a stable operation could be established for droplet volumes from 0.1 to 0.7 nl (Fig. 7).

The risk of cross-contamination was investigated by filling the printhead with water and black ink in a checkerboard pattern. Any cross-contamination would result in traces of the dye in the water spots. First tests were made with elastomer-stamps of type A (see Fig. 8). These original flat shaped stamps clearly showed problems. Nearly every stamp insertion into the printhead led to cross-contamination. The supply channels are open where they lead to the nozzles (see Fig. 6). When placing the flat rubber stamp it can happen that fluid is drawn in the tapered slit between the

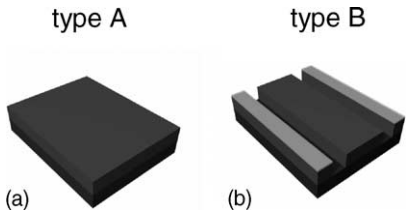


Fig. 8. Investigated stamp geometries: (a) flat elastomer-stamp; (b) spacer directly adjacent to the rubber stamp.

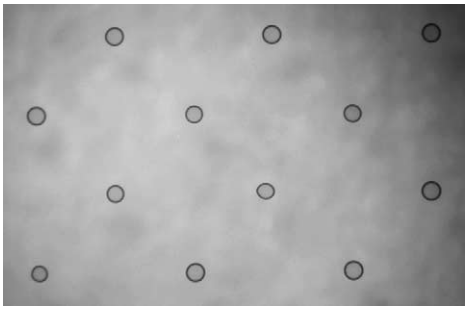

Fig. 9. Water and black ink are printed in a checkerboard pattern to demonstrate the absence of cross-contamination.

print window and the stamp surface by capillary forces, causing cross-contamination. A solution to this is to guarantee a complete parallel approach of the two surfaces. This can for instance be achieved by using special stamp geometries like those of type B as shown in Fig. 8. They consist of one middle elastomer part, which covers the nozzle array and two outer, elevated pieces. These flanking parts prevent a contact between the middle part and the nozzle array during stamp insertion. The outside rim of the stamp will first touch the printhead inside where there are no supply channels, and so align the rest of the surface. If pre-stress is applied, the middle part is lowered onto the nozzle array in a controlled parallel way. With that cross-contamination could be prevented and the expected print result without cross-contamination could be observed as shown in Fig. 9.

## 4. Conclusion

Fig. 7 shows that the droplet volume for a parallel ejection of 24 droplets can be precisely controlled by the piezo-stack actuation over a range of 100–700 pl. Droplets as small as 60 pl and as high as 800 pl were also generated with a different stamp design and material. The new concept has a clear linear relation between actuation amplitude and dispensed volume. Additionally, stable operation can be achieved by controlling piezo-stack velocity.

The two parameters, piezo stroke and piezo speed are not fully independent. Droplets with different volumes show different speeds even if piezo speed is held constant. The piezo speed has to be controlled to adjust droplet speed for stable operation. If the provided actuation energy is too high, the droplet speed gets too high and satellite droplets appear. If it is too low no droplet or not all droplets tear off clearly. This means that for every desired droplet size there is a set of parameters that work problem free.

The droplets in one array show different speeds and volumes in a specific pattern which is dependent on the nozzle array design. Despite of the observed pattern a stable operation was possible at a droplet volume range of 0.1–0.7 nl, whereby the droplet volumes in one row show a standard deviation below 5%.

The elastomer-stamp must have a certain shape to prevent cross-contamination during its insertion into the printhead. It was shown that it is possible to place the special shaped rubber stamp into the printhead without causing cross-contamination. Measurements with fluorescence dyes have to be performed to get more precise results than those with water and ink.

The complete control of the droplet volume signifies that we can control the actual spot size in the microarray. The first results also showed that this droplet volume (and thus the spotsize) is independent of the dispensed liquid. This significantly enhances the flexibility in microarray production.

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