A 96 Channel Printhead
For Production Of Microarrays

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Abstract

A 96 channel printhead for simultaneous delivery of 96 different biochemical substances in nanoliter quantities was built and tested. The printhead consists of two Pyrex layers that sandwich a Silicon wafer. The silicon was structured using Deep Reactive Ion Etching (DRIE). Critical points in the fabrication were identified and solutions presented. The final result is a printhead that works for a broad range of liquids and enables a far higher throughput in the production of biochips/microarrays as compared to currently available systems.

Keywords: Microarrays, biochips, drop on demand, DRIE, capillary channels, nozzles, nano-liter dosage

1. INTRODUCTION

Micro-arrays can be regarded as high throughput analytical instruments. The basic idea behind the concept of microarrays is very simple; many different analytes are placed (immobilized) in a regular arrangement on a substrate. The substrate with the regular array of immobilized analytes is called microarray, biochip or DNA-chip. Depending on application, some hundreds up to some 10.000 different analytes are used on one single substrate. An unknown sample liquid is brought into contact with the microarray. If there are ingredients in the sample liquid which are complementary to a certain immobilized analyte on the microarray, these ingredients will bind to that analyte. The absence or presence of binding events can be detected by e.g. fluorescence. In this way an unknown sample can be screened towards many different potential ingredients in one single process.

Microarrays are slowly moving from a strict research tools to mass-market products. Connected with this is a rising demand for industrial scale production tools. The currently available techniques for fabrication of microarrays can be divided in contact and non-contact techniques. From the application point of view the non-contact techniques have some clear advantages over the contact techniques. Therefore the non-contact array printers are gaining popularity but also these have a number of limitations. The main draw-back in most systems is the limited ability to change from the well plate format (in which the probes are normally delivered) to the micro array format. The TopSpot technology presented in this paper is one of the very few tools that allows high-throughput production of low and medium density microarrays (up to some thousands of analytes per substrate).

For some time we have been developing the TopSpot technology for production of micro-arrays [1,2]. The print technique uses silicon micromachined printheads with many nozzles placed at the micro-array format. Recent developments include a 96-channel printhead for high throughput production. The TopSpot machine consists of a silicon printhead, placed in module that holds the actuator and a slider for positioning the substrates.

2. DESIGN

The printhead consists of integrated reservoirs which hold sufficient liquid for 1000 to several thousand prints (see Fig. 1). These reservoirs are connected with the ejection nozzles over capillary channels, which also provide the format change. For ease of handling the reservoirs are placed on the pitch of a conventional wellplates. This gives the possibility to fill the reservoirs using commercialy available pipetting-robots. The nozzles are arranged on the pitch of the microarray (typ. 500µm). Currently chips hold either 4x24 or 8x12 nozzles in one array.

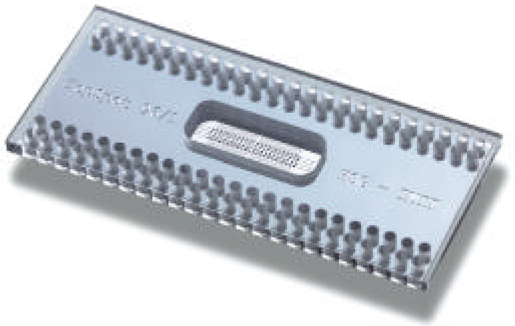


Fig. 1 View of the 96 channel printhead.

2.1. The Nozzles

The ejection of droplets is comparable with that used in ink-jet printers. A pressure pulse is applied to the liquid on one side. The pressure will give an impulse to the liquid in the nozzle which in turn causes a small droplet to break through the surface tension. The pressure pulse is generated on the topside of the nozzle channel (see fig. 2) by compressing an air chamber. To guarantee reproducible and uniform droplets the nozzles need to be of the same size.

Micro-fabrication techniques allow for a very high geometric precision. The most important dimension is the nozzle diameter which is defined with photo-lithographic techniques. This gives the highest possible accuracy. The length of the actual nozzle channel is less critical, but also here a constant etch depth is guaranteed through constant etch parameters.

2.2. The Capillary Channel

The capillary channels have two functions. First and foremost the transport of the liquids from the reservoirs to the nozzles.

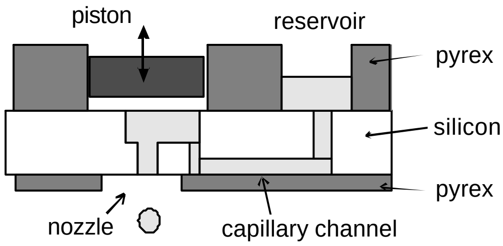


Fig. 2 Schematic cross-section of the printhead.

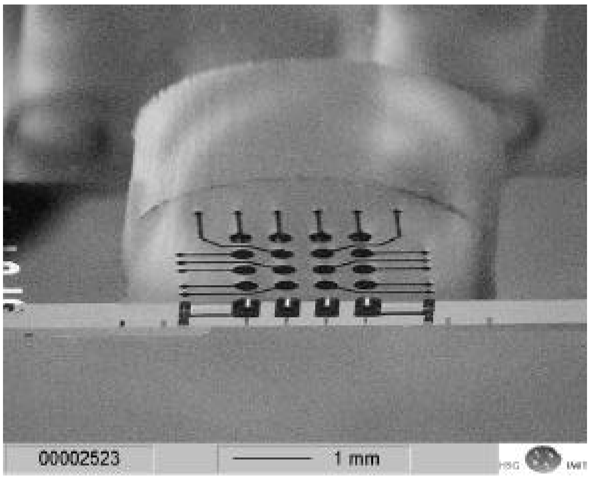


Fig. 3 Cut through a fabricated chip. Schematic is given in Fig. 1

Not only with the filling but also after every droplet ejection to refill the nozzle channel. The driving force is the capillary force of the liquid in the channel. Characteristic for this is the contact angel of the liquid with the material of the channel wall. The smaller the contact angle the higher the capillary pressure and thus the capillary flow rate. The second function of the channels is the connection and thus the format change of the 2.25mm pitch of the reservoirs to the 500µm pitch of the ejection nozzles. The maximum complexity of a printhead is limited by the space available to place and cross channels.

The changes in diameter of the channel are the critical points in the system. Ideally there are no sharp changes but due to the limitations of silicon micro-machining this is not always possible. At points where a diameter change is necessary it is important to guarantee that at least one point of continues-channel-wall [3]. Special care needs to be taken here in view of the fabrication tolerances. Either by designing the connecting layers such that the continues wall is always possible or by using critical parts of the mask only from one layer (see also 2.4 Fabrication).

2.3. Actuation

Instead of separate actuators for each channel we use only one actuator for all 96 channels. This improves the reliability and makes a far simpler system. The actual actuator is a piezo-stack of 80mm length. This actuator moves a piston that is used to close off an air volume above the nozzle channels. (see fig. 4) The o-ring that is used to close off the air volume works also as a spring to keep the piston in contact with the piezo actuator.

A small hole is provided in the piston to equilibrate the pressure after the actuation. When the piston moves back too fast a vacuum might be created in the pressure chamber. This could cause small droplets to be ejected inside the pressure chamber (similar to the ejection mechanism at the front side), causing cross-contamination. Slowing the piston on the backwards motion together with the equilibrium hole prevent this from happening. The actual size of the ejected droplets can be influenced by the initial height of the pressure chamber and the dynamics of the piezo actuator.

2.4. Fabrication

The printhead consists of three layers (see Fig. 3 and 4). The two Pyrex layers are structured using ultrasonic machining. The silicon layer, that holds all the relevant fluidic structures, is structured using Deep Reactive Ion etching (DRIE) (See fig. 5). From both sides of the wafer two etch steps are necessary to arrive at the desired geometry. Both sides use a combination of a silicon-oxide and a photoresist masking layer.

First, from the nozzle side the second etching mask is transferred in an oxide layer. The oxide thickness is calculated from an etching selectivity against Si of 60. Secondly the photoresist is applied and structured, for the first etch step.

In this first etch step the nozzles, via´s and

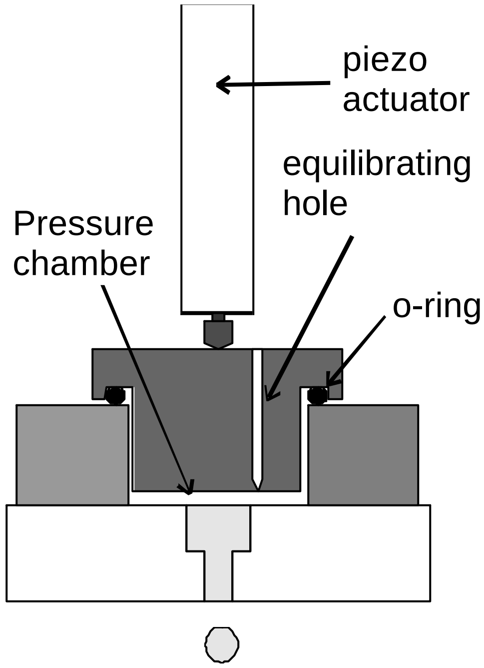
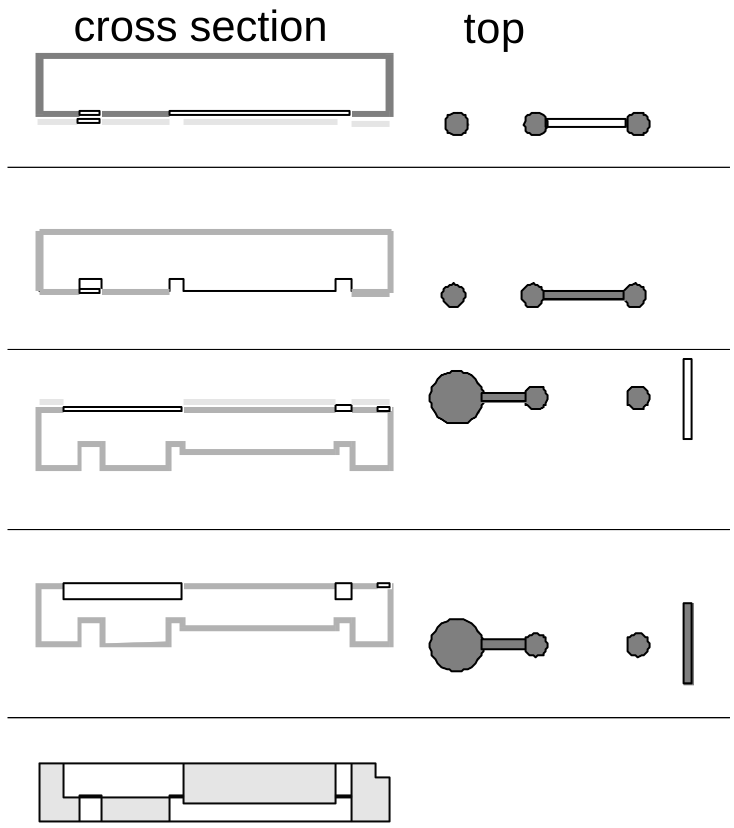


Fig. 4 The piezo actuator and the piston with equilibrating hole



1) An oxide layer is grown and structured.

2) Photo resist is removed and the second etch step

3) Oxide protection layer is structured. Photoresist covers those parts that are only etched in the second step.

4) Resist is removed and the connection to bottom side is made.

5) Final result.

Fig. 5 Processing of the silicon wafer,
left the cross section right, the different masks.

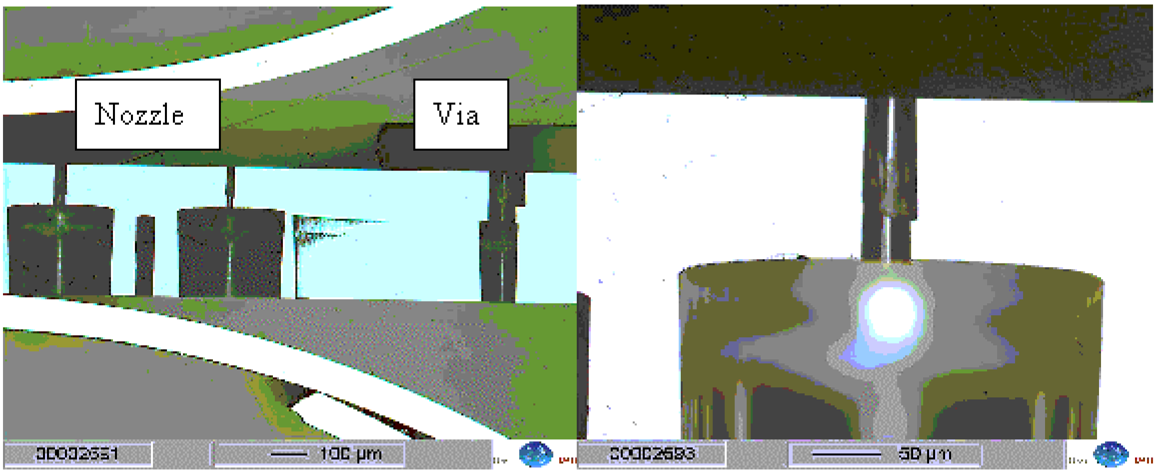


Fig. 6 Cross section of actual fabricated devices. Left both the via (left in the image) and right the nozzle.

reservoir connections are etched for 50µm. After ashing the remaining resist, the second etch step is made (100µm deep), to arrive at connection channels of 100µm deep and nozzles, via´s and reservoir connections of 150µm deep.

All oxide is stripped and a new protection layer of oxide is thermally grown. This oxide layer serves also as masking layer for the second etch step from the top side. The first etch mask is again a photoresist. In the first step the connection channels, the nozzle topsides and reservoir connections are etched. The second step includes also the dicing marks and the text on the chip. At break through the oxide layer protect the backside cooling gas from entering the process chamber. The wafer is now cleaned and the oxide stripped. To improve the hydrophilic properties a final oxide layer is grown (100nm). The bonding is done at wafer level after which chips are diced.

Finally the chips get cleaned in an oxygen-plasma and the nozzle side of the chip receives a hydrophobic coating.

3. RESULTS AND DISCUSSION

The connection of the different layers to each other is crucial for the fluidic properties of the printhead. To check the etching results several printheads were cut to get a view of the cross-section. These etch results were compared with the filling properties to gain insight into the defect tolerance of the system.

3.1. Micromachining Results

The connection of the first to the second layer of the nozzle side etching turned out to be crucial. In some misalignment cases a rim occurs that hinders the fluid meniscus during filling (see fig. 6). By using the oxide layer for both etch steps we hope to improve on this situation.

Also the nozzle slightly suffered from this alignment problem. Also here improvements can be expected by using the oxide layer for both etch steps.

3.2. Self-filling Properties

Water has a high surface tension, this means that small perturbations in the channel geometry could cause the filling of

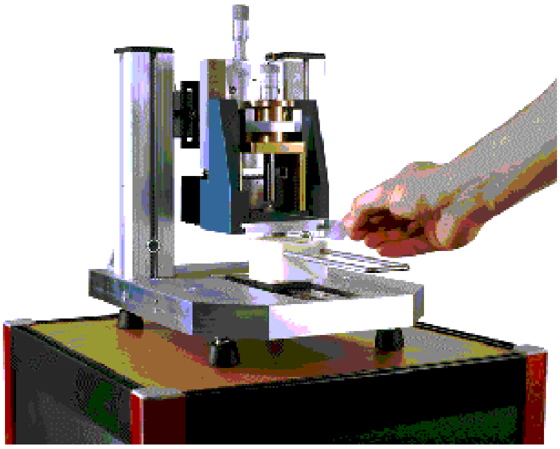


Fig. 7 The print module that guarantees constant printing conditions.

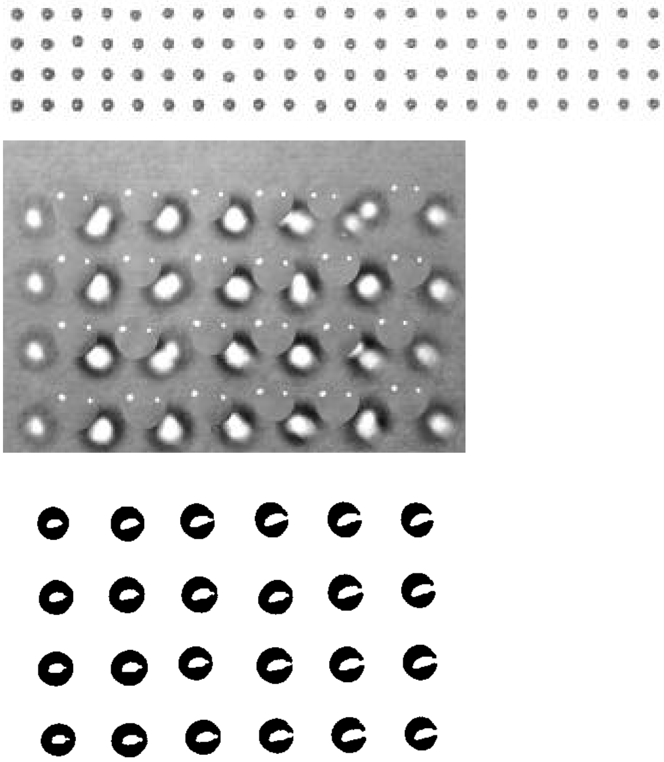


Fig. 8 Typical print results, top to bottom: 96 channel print with water, 24-channels with DMSO, 24-channels with NaCl buffer.

one channel to stop. From a representative batch of chips (60 chips) we measured the self filling properties and got the following results: Only 4% of the chips showed minor problems with the filling. We expect this number to improve with the mentioned changes in the masking.

3.3. Printing

Printing was tested not only with water but with a number of other liquids. To get reproducible print results a print module was built . The printmodule hold a kind of drawer that positions the printhead (See fig. 7). The piston with its bearing is connected to this drawer such that after each chip change the position of the piston is guaranteed. To minimize the evaporation of liquid from the chip the drawer can be cooled. A slider is used to position the test substrates under the print head.

For brevity only some typical print results are shown (Fig 8.). Apart from water we dispensed a wide variety of liquids; DMSO, NaCl Buffer, but also glycerol/water mixtures (up to 50% by weight).

From printing tests with water we found an in array (selection of the 96channels) standard deviation of 3µm with a spot diameter of 210µm (see fig. 9). The standard deviation of the average sizes between different runs was found to be much smaller then the 'in-run' deviation (1.4 µm).

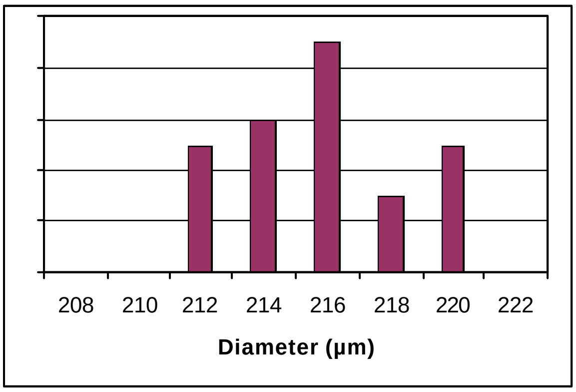


Fig. 9 Plot of the measured spot diameters.

An other good measure for the stable operation of the printhead is the observation of the droplet detachment. Different parameters of the printmodule influence the droplet detachment. Optimum parameter give satellite free operation (see fig. 10)

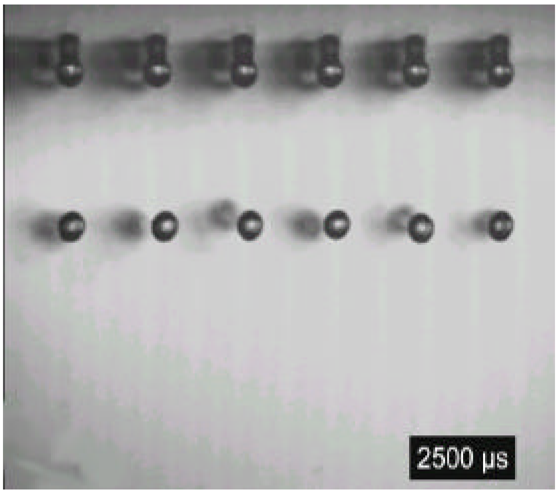


Fig. 10 Drop detachment.

4. CONCLUSIONS

We managed to build a 96 channel printhead that has a high reliability and repeatability. Minor problems in the filling were localised to the connections of the different etch steps.

The printing result showed that it is possible to deliver a wide variety of liquids without problems. With this system it is for the first time possible to produce micro-arrays at an industrial scale.

5. ACKNOWLEDGEMENTS

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