LAMINAR FLOW MICROARRAY PATTERNING BY PERPENDICULAR ELECTROKINETIC FOCUSING

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ABSTRACT

This paper describes a method to pattern microarrays in a closed microfluidic device. Two perpendicular laminar flow streams can operate in terms to sequentially coat the surface of a flow-chamber with parallel lanes in two directions. Two perpendicular sample streams can be controlled in position and width by applying electrokinetic focusing, for which each of the two streams is sandwiched between two parallel laminar flow streams containing just a buffer solution. Electroosmotic flow allows a simple chip design without any moving parts being involved. With this device configuration it is possible to define an array of up to 169 spots on a surface area of 1 mm^2.

Keywords: electrokinetic focusing, electroosmotic flow, laminar flow, microarray

1. INTRODUCTION

Microfluidic offers new physical environments such as laminar flow, characterized by low Reynolds numbers. The position and the width of a laminar flow stream can be varied by sandwiching the stream between two parallel sheath flow streams. This technique, in case of pressure driven systems, is known as hydrodynamic focusing. Hydrodynamic focusing has been applied for patterning metal and polyelectrolyte structures inside microfluidic channels [1] and biochemical arrays on microfluidic chips. Parallel lanes of immobilized proteins could be patterned in one operation step. For a more complex geometry like an array, the device had to be removed from the substrate and turned 90 degrees for the second immobilization [2, 3]. Electrokinetic focusing is similar to hydrodynamic focusing but with electroosmotic flow instead of pressure driven flows. It is straightforward to control parallel fluid streams in position and width inside a laminar flow chamber using electrokinetic focusing [4].

The device described here uses electrokinetic focusing with two perpendicular laminar flow streams for an adjustable patterning of bio-chemical assays. Since it is a closed and liquid filled chip, both streams can operated in terms without the array spots being exposed to air.

2. PRINCIPLE

Each chip consists of six inlet channels connected to a wider flow-chamber and two outlet channels. As shown in figure 1, during the first patterning step (a), three inlet channels are active and fluid is pumped through them by electroosmotic flow (EOF) while the perpendicular channels are passive. The position and the width of sample stream 1 entering the flow-chamber can be controlled by focusing with two parallel sheath flow streams. The sample stream is guided to the desired positions to sequentially immobilize lanes of e.g. proteins inside the flow chamber. Sample stream 2 is then (b) used to immobilize lanes in the perpendicular direction. If appropriate surface and bio-chemistry is applied, this patterning procedure results in a micro array with the number of spots based on

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the stream widths and spacing used. The voltages required to generate the adequate electro-osmotic flows, depending on velocity, positions and stream widths are calculated real-time with an analytical model. This model is an analogy of electroosmotic flow to electrical circuits.

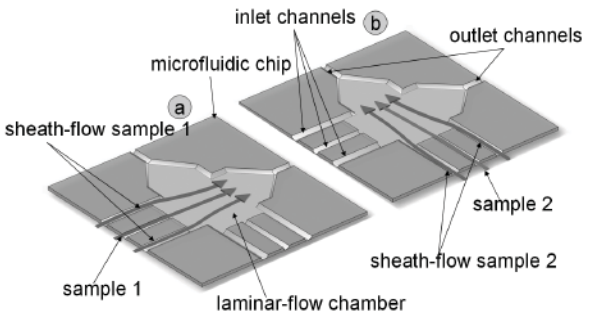

Figure 1. Two perpendicular sample streams operate in terms to immobilize lanes of molecules.

3. FABRICATION

As illustrated in figure 2 standard cleanroom fabrication techniques were applied. Each chip (15x15 mm^2) consists of two plates. The silicon top plate contains the microfluidic channels as well as inlet and outlet holes while the bottom Pyrex glass plate is left unprocessed. The 10 µm deep channels and chamber were etched into the silicon with a first reactive ion etching (DRIE) step. The pyramidal shaped inlet and outlet holes were etched through the wafer, starting from the backside in a KOH isotropic wet etching bath. After removing the etching masks and photoresist, the structured silicon wafer was oxidized to build up a 1 µm thick layer of silicon oxide. This layer serves as an electrical insulator to prevent short circuits while applying the voltages required for EOF.

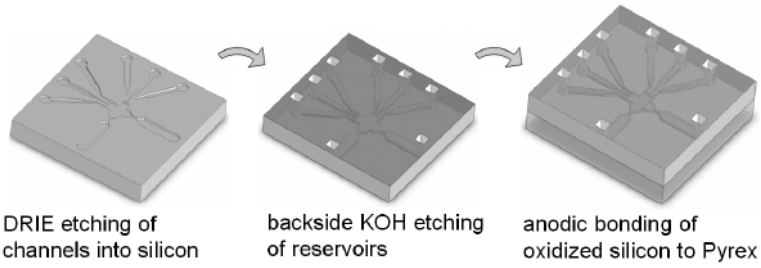

Figure 2. Main fabrication steps for the micro patterning device

4. EXPERIMENTAL

The chip was placed in an in-house fabricated holder containing eight reservoirs with integrated platinum electrodes connected to two high voltage sources (IBIS 411, microfluidic control unit, IBIS Technologies B.V., the Netherlands). The applied voltages were controlled with a LabView (National Instruments) program based on an analytical model. An inverted microscope (Olympus IX51) with fluorescence unit, equipped with an F-View II 12-bit digital camera was used to view the stream profiles. All inlet reservoirs and the connected chip were filled with 10 mM HEPES in demineralized water (pH 7.4). During experiments fluorescently labeled bovine serum albumin (BSA) (0.5 mg/ml in HEPES buffer) was added as a stream marker to the sample stream reservoirs. Throughout microfluidic operation, microscopy images were captured using the camera control

software AnalySIS (Soft Imaging System GmbH, Germany). To visualize and study the array geometry the captured images of single streams were merged to one overlay image.

5. RESULTS AND DISCUSSION

As seen in figure 3 it was possible to control the position of the two perpendicular sample streams by electrokinetic focusing to create a microarray. This figure shows micro arrays with 25 and 169 spots. The single images were combined to one overlay using photo editing software. Due to the cross shaped flow chamber geometry the stream lines tend to diverge in the middle part. This effect results in a round shaped array geometry and non uniform shaped spots.

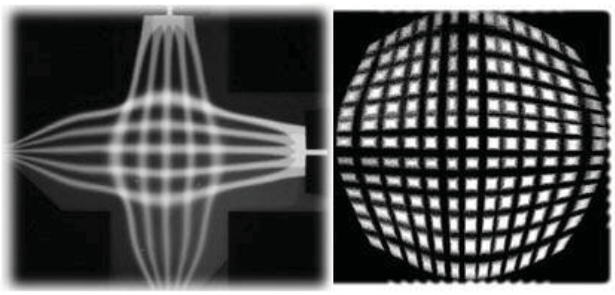

Figure 3. Image overlays visualizing possible array geometry: left: 5x5 streams, right: 13x13 streams with (only the overlapping areas made visible) and a spot size of ~25x25μm^2

6. CONCLUSIONS

A microfluidic device for the immobilization of biomarkers to form an array has been developed and experimentally characterized. Two electrokinetically focused sample streams can be positioned inside a laminar flow chamber by controlling only the applied voltages. Parallel lanes of molecules can be immobilized in two directions while the overlapping areas form the array spots. The principle has initially been demonstrated with fluorescein as a stream marker and visualized with picture overlays. Experiments with protein arrays are underway. Further investigations focus on the influence of proteins on the chip surface properties and therefore on the electroosmotic flow.

ACKNOWLEDGEMENTS

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