TOPSPOT- A NEW METHOD FOR THE FABRICATION OF MICROARRAYS

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ABSTRACT

This article outlines the new non-contact TOPSPOT method for printing microarrays in a highly parallelized fashion. It is based on a micromachined print head incorporating a central microarray of presently up to 96 vertical nozzles on the output side. Droplets featuring volumes down to 1nl aligned in a 500µm grid are simultaneously ejected by applying a steep air pressure ramp to the open upper side of the liquid.

Each of these nozzles is connected to a distinct fluidic reservoir constituting the interface to the macro world. To allow an automated replenishing of the chip, the alignment of the reservoirs is amenable for liquid handling via standard pipetting robots. Depending on the design of the print head, a maximum of 20µl can be stored thus allowing to dispend about 20.000 droplets of equal quality in a row. Besides its suitability for a robust biochip manufacturing facility in the laboratory, the print head is also well-integrable in a high-throughput production plant. These versions are scheduled to become commercially available in 2000.

INTRODUCTION

Biochips (Fig. 1, bottom part) hosting arrays of microdots are deemed to become one of the key analytical tools in life science. The range of possible applications comprises low-cost chips for cheap on-site analysis to drug testing in pharmacogenetical research. Provided that research will manage to develop practicable solutions concerning the biochemistry, the generation of tightly spaced arrays of nanoliter-droplets, the immobilization of target molecules on the surface and data read-out, biochip technology is forecast to conquer a high-volume market [1].

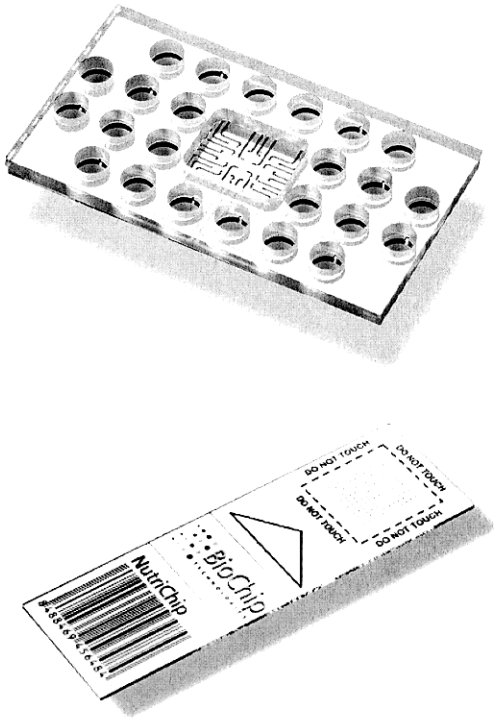

Fig. 1. (top) TOPSPOT print head. Our proprietary 24-nozzle chip is used for printing microarrays. **(bottom) Example of a biochip**. This product named NutriChip™ for food analysis is commercialized by BioChip Technologies, Germany.

This paper focuses on one of these pivotal issues – the arraying of small liquid droplets on solid surfaces like glass slides or nylon membranes. Despite tremendous research

activities in this field, only partial solutions have been available so far: (i) ink-jet-spotting [2], (ii) contact printing [3] and (iii) on chip synthesis [4]. In our eyes, all of them display severe deficiencies concerning reliability, speed, precision, wear or the volume stored in their liquid reservoirs. At present, there is no machine on the market complying with the requirements of a high-throughput industrial production of biochips each hosting a variety of several hundred analytes.

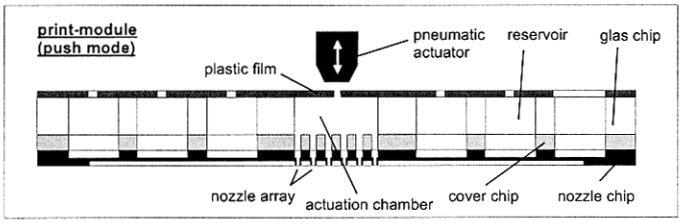

Fig. 2. Design of the TOPSPOT print head. The upper side of the part featuring the capillary system is displayed in more detail in Fig. 3, the nozzle array on the bottom side in Fig. 4.

OUTLINE OF THE TOPSPOT PRINCIPLE

Addressing the industrial demand for such a high-throughput device targeted at the low-cost production of microarrays at medium densities (up to 1000 droplets per chip), HSG-IMIT has recently developed the so called TOPSPOT technology [5,6]. Much like in ink-jet printing, droplets are dispensed from a multi-nozzle cartridge (Fig. 1, upper part) on a surface after passing a small air gap of about 500µm. This simple method proves to work reliably for a broad rheological range of substances.

The print head consists of two silicon wafers and a Pyrex wafer which are microstructured by dry etching and then stacked by silicon fusion and anodic bonding, respectively (Fig. 2). In a central array on the lowermost chip (Fig. 3), the nozzles are etched through the full wafer depth. Each of the nozzle tips is structured from the backside (Fig. 4).

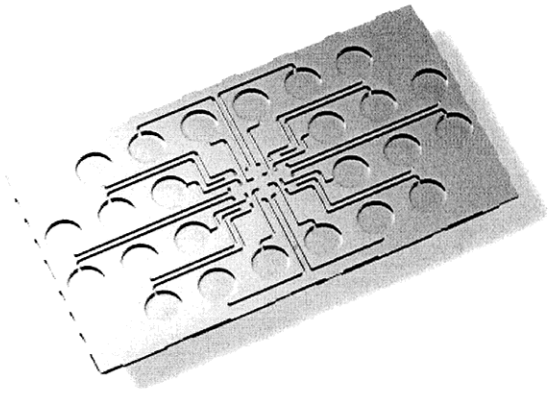

Fig. 3. Top view of the silicon chip on the bottom side of the 24-nozzle print head. The horizontal capillaries connect the fluidic reservoirs with the central nozzle array.

The spacing of the nozzles corresponds to the lattice constant of the microarray to allow high-quality printing of droplet arrays in a massively parallel fashion. With a grid spacing of 500µm and repetitive array spotting on one chip, a variety of several thousand different analytes can be accommodated on a single standard microscope slide.

The interface to the macro world, i.e. standard liquid handling robots, is realized by the fluid reservoirs aligned according to standard microtiter plate spacing on the upper side of the print head (see also Fig. 1 and 2). The TOPSPOT module therefore features a so-far unmatched parallelism on the input as well as on the output side which is viable for high-speed production of biochips.

DESIGN OF THE TOPSPOT PRINT HEAD

On the top side of the chip, capillaries connect each nozzle with their distinct fluid tank. The grid distance corresponds to the 384- and 1536-microtiter plate formats for the 24- and 96-nozzle heads, respectively. In the latter case (Fig. 5), two compact 48-well subgrids have been formed which can quickly and conveniently be filled by parallelized standard robots. With appropriate automation which is commercially available, all reservoirs may ideally be filled in two pipetting cycles only.

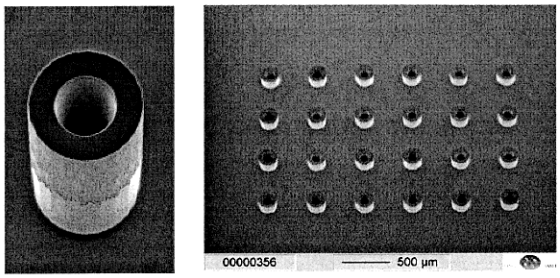

Fig. 4. Bottom view of the nozzle array.

The capillaries are sealed by the second silicon wafer featuring full-depth blank spaces above the reservoirs and the nozzles. In order to increase the capacity of fluid storage, the silicon reservoirs are furthermore extended by appropriate holes in the upper Pyrex wafer. (In the print head version of Fig. 1, the central silicon layer is missing. The capillary system is directly covered by the Pyrex wafer.) Since the pneumatic actuation described in the following section requires an air buffer above the nozzle array, a central window is cut into the Pyrex above the microarray by ultrasonic drilling. The print head is covered by a plastic foil to avoid evaporation of the reservoirs. Small holes in the foil allow for a relaxation of the pressure gradient above the liquid compartments of the print head.

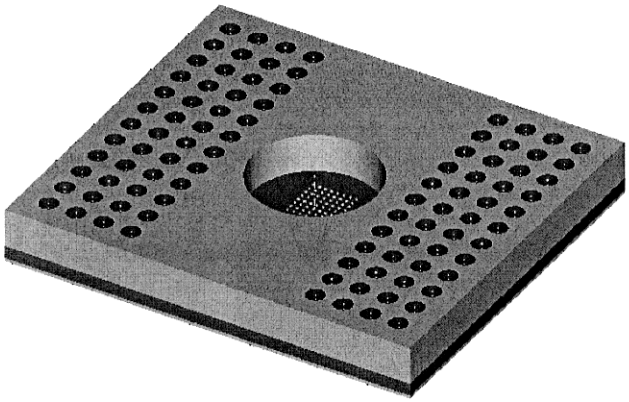

Fig. 5. Layout of the 96-nozzle print head.

GENERATION OF DROPLETS

After filling the reservoirs, the nozzles are moistened by mere capillary forces. The liquid columns in the nozzles are sustained by surface tension.

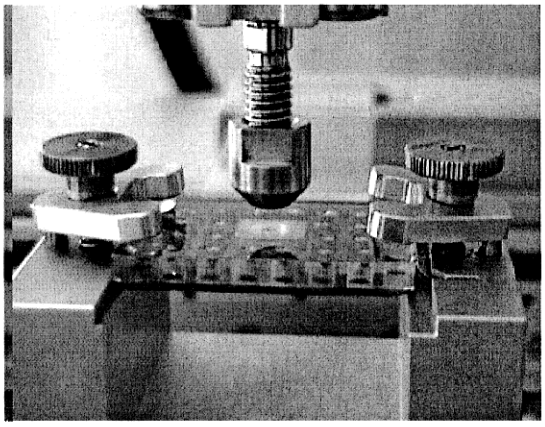

Fig. 6. Actuation of the TOPSPOT module by a pneumatically driven piston denting the plastic foil.

The plastic cover also provides a compressible actuation chamber in the central Pyrex window above the nozzle array. Droplet ejection is triggered by a pneumatically actuated piston denting the foil to compress the air above the open ended liquid columns (Fig. 2 and 6). Thus, the same pressure gradient is applied to all nozzles driving a fluid plug out off the bottom side of the chip. If surface tension is overcome by inertia, the liquid escapes from the nozzles forming nanoliter droplets (Fig. 7).

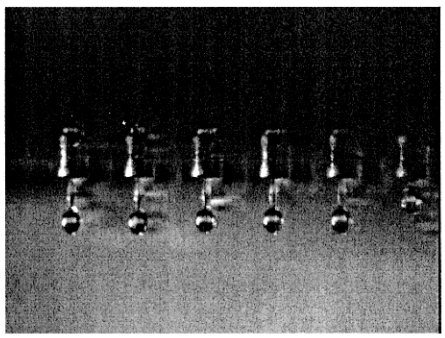

Fig. 7. Droplet formation.

This simple mechanism appears to widely eliminate the well-known sensitivity of the ink-jet printing to liquid properties like viscosity and surface tension. Furthermore, TOPSPOT renounces on the addressability of single nozzles which is obsolete in a biochip production line. Instead, the same pressure ramp is collectively applied to the whole ensemble of nozzles leading to the simultaneous ejection of all droplets.

The new TOPSPOT principle also simplifies the mechanical setup compared to our initially tested actuation principle which

implements a piezo-bimorph lever literally shaking the droplets out of the print head (Fig. 8) [7].

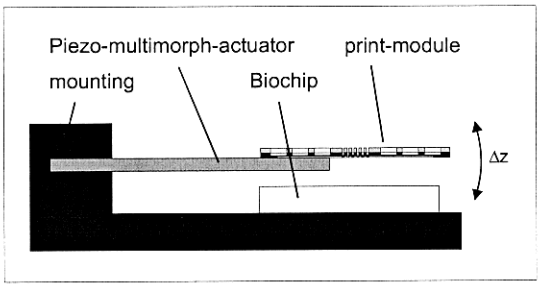

Fig. 8. Piezo-bimorph actuation of the print head.

Despite its proven robustness for the 24-well module, this formerly used method also lacks scalability because the maximum acceleration is obviously limited by the weight of the print head. Additionally, the freedom of the chip layout is restricted by enhanced requirements concerning the mechanical stability.

PRELIMINARY RESULTS

The quality and reliability of our TOPSPOT printer has been verified by qualitative means so far. Putting the print head into operation proves to be quite uncomplicated. After replenishing the reservoirs, printing results already stabilize after the first droplet array. Fig. 9 depicts droplets spotted in 3x4 grid with the 24-nozzle TOPSPOT module on a silanized glass slide. Each row contains different biological substances, from top to bottom a PCR-product (no purification,~1g/L), lambda-DNA (~0.5g/L), plasmid-pKS (~0.7g/L), standard oligos Ct40 (100µM), Ct38 (100µM) and short oligos (~100µM), diluted in aqueous solution.

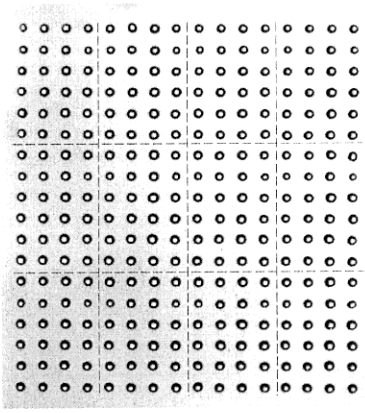

Fig. 9. Exemplary droplet array printed with a 24-nozzle TOPSPOT module.

The reliability for droplet ejection is very high. In fact, no clogging or sudden failure during print cycles has been discovered. From visual inspection of the droplets before evaporation, we found slightly diminishing droplet diameters on the surface for increasing viscosities of the substance. A verification of the droplet volume via fluorescence scanners is needed for an improved quantification.

For a certain substance, the droplet volume does not significantly vary with the spatial amplitude of the actuation rod. Typical droplet diameters in the liquid phase primarily depend on the nozzle diameter which they typically exceed by about 20%. Print heads with nozzle diameters down to 100µm and featuring array spacings down to 500µm show constant-quality performance. Nozzle diameters down to 50µm leading to droplets of about 60µm diameter are currently under investigation. With silanized glass slides and sporadic tests on other surface species, no donut shapes of the droplets have been observed which frequently occur when employing pin-printing devices.

COMMERCIAL IMPLEMENTATION

In the rapidly evolving biochip business, there is a strong request for reliable microarraying machines. On the one hand, many laboratories are still in an explorative stage of their proprietary biochip technology. For their purposes, compact, moderately prized microarrayers for low-volume production of medium-density biochips are demanded. In this market segment, our so-called "Basic Microarray Workstation" and "Multi-Head Microarray Workstation" presented in the following compete with a number of contact and ink-jet printers which are already commercially available.

On the other hand, some companies possess ready-to-go solutions requiring high-throughput production of low-cost biochips. To our knowledge, no product is currently offered to serve this business segment which, in our view, displays a tremendous growth potential within the next years. Our "Microarray Production Plant" introduced at the end of this section envisions this interesting market.

Basic Microarray Workstation

Glass slides are inserted into a drawer underneath the print head. A mechanical stop assures correct alignment of the slides with respect to the print head. A lateral offset can be applied to the drawer by two perpendicular micrometer bolts. This way, multiple prints can be performed to spot adjacent arrays on the same slide.

The print heads can either be filled manually or by standard liquid handling robots. Considering the high susceptibility to errors while handling a large manifold of different media, the latter automated solution is preferable, in particular if the same

set of print heads is frequently exchanged and filled with a different set of media. The print heads are then covered by the foil, placed in a designated holder and locked. For each printout, the pneumatic actuation is triggered by pressing a button.

The very robust Basic Microarray Workstation provides a competitive microarray facility concerning the quality of printouts compared to state-of-the-art technologies. In terms of time for the fabrication of a biochip, the parallelism of this research machine implies another decisive advantage since, even in completely manual operation, the once filled print head may easily deliver identical 24- or 96-dot printouts at an approximate rate of five per minute which is limited by fully manual operation. Commercial versions of this biochip workbench will be available in spring 2000.

Multi-Head Microarray Workstation

For a small series production of biochips, a further automation step has been introduced in the so-called Multi-Head Microarray Workstation (Fig. 10). Slides can be attached to an xy-table. Computer control can be programmed to subsequently align the carriers underneath the print heads and to trigger the actuation of each print head. A maximum of 10 individual print heads can be mounted simultaneously on this workstation. This way, up to 960 different liquids (10 print heads with 96 different nozzles) can be dispensed on the carrier slides. The quality of each printout is automatically surveyed by a CCD-camera which is linked to an image capture and processing software.

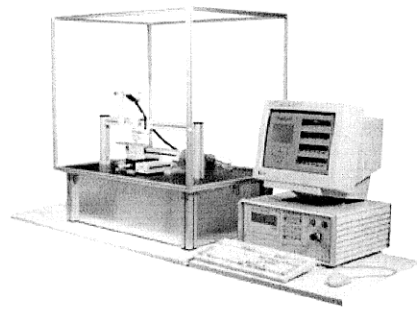

Fig. 10. Design of the semi-automated "Multi-Head Microarray Workstation". Slides are handled by an computer-controlled xy-table. Quality control is performed by an integrated image processing.

With an optional liquid handling automation, climate chamber and exchange of print heads, this compact machine yields a convenient access to small-scale biochip fabrication.

Microarray Production Plant

To accomplish a high-volume fabrication for medium-density biochips, the concept of a fully automated production line featuring five pairs of 96-nozzle print heads has been elaborated by the Fraunhofer-IPA located in Stuttgart (Fig. 11). In a single run, 960 different media can thus be spotted on each slide.

Sets of carrier slides are stored in magazines which are delivered to the print stations by a conveyor-belt. Two carousels temporarily store incoming and outgoing magazines. Microclimate chambers assure constant quality of the printouts. A robot arm takes single carries out of the magazines and submits them to the double-print heads at each of the five stages. At the end of the production line, an image processor verifies the quality of the biochips and sorts out improper carriers. A barcode system identifies print heads and slides to monitor the production process in a software environment.

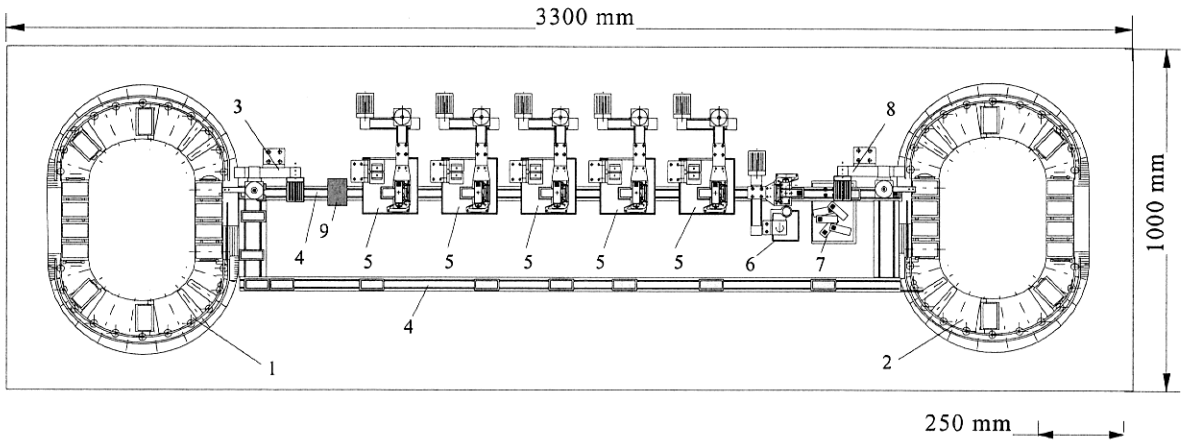

Fig. 11. Concept of a fully automated biochip production line featuring five pairs of print modules and quality control.

Assuming a duty-cycle of about 10 seconds per double-headed print station, a throughput of nearly 3000 biochips per 8-hour shift can be realized. Due to the large reservoir volume, 5µl or 20 µl depending of the print head design, several shifts can be run without the need to replenish the print heads. Our production machine is planed to start operation in summer of 2000.

CONCLUSIONS

In this article, we introduced our proprietary TOPSPOT technology for spotting microarrays. This novel principle excels due to its simple and robust pneumatic actuation and its presently unmatched, up to 96-fold parallelism on the input and output side.

Three concepts have been presented for incorporating the TOPSPOT technology into commercial biochip facilities. While the manually operated "Basic Microarray Workstation" and the semi-automated "Multi-Head Microarray Workstation" are primarily designed for in-house and small-volume fabrication, the "Microarray Production Plant" displaying a fully automated supply chain targets the still emerging market for high-throughput production of low-cost biochips.

ACKNOWLEDGMENT

The TopSpot-Printer has been developed in cooperation with the Freiburg-based company BioChip Technologies and HSG-IMIT and was partially funded by the "Zukunftsoffensive der Landesregierung Baden-Württemberg, Förderung der Biotechnologie". The detailed evaluation of a multi-module production machine results from a corporation with the Fraunhofer-Institut für Produktionstechnik und Automatisierung (FhG-IPA).

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