Fabrication of microarrays on an industrial scale with topspot

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Abstract TopSpot technology allows the printing of microarrays on an industrial level. It is based on a micromachined print head incorporating presently an array of 96 nozzles placed at 500µm grid distance. Droplets in the nl-range are ejected simultaneously and on demand by a pressure pulse applied to the open upper side of the nozzle array. The droplets hit a carrier slide and subsequently evaporate to form a microarray of dried biological substances like DNA fragments

Refilling of the nozzles takes place through microchannels which are connected to separate reservoirs on the upper side of the print head. The spacing of these reservoirs corresponds to microtiter plate format to provide a convenient interface to standard laboratory liquid handling robots. With a storing capacity of some µl of each reservoir, thousands of identical slides can be printed. Outputs of 5,000 slides each carrying several 100 different analytes, which are dispensed by repetitive printing, are possible. Partially and fully automated devices incorporating TopSpot print heads will be available in 2000.

Introduction

For economic reasons, large pharmaceutical companies are expected to present new drugs each year. The quest for new drugs has fostered automated "shotgun" approaches commonly referred to as combinatorial chemistry. Large libraries of drug candidates result from the arbitrary combination of smaller compounds. Nowadays, standardised microtiter plates with 96 wells, in combination with pipetting and dispensing robots, are the method of choice for generating these combinatorial libraries. Further miniaturization via 384- and 1536-well plates with well-spacing of a few mm only is already available. Most experts agree that this will be the ultimate barrier for the microtiter plate concept.

Microarrays, also referred to as biochips, are commonly considered key to pharmaceutical research due to their potential for high-throughput analysis. In a certain sense, microarrays can be regarded as well-less microtiter plates where liquid volumes are confined by surface tension or adhesion/bonding to the carrier. In this way, integration can be enhanced by some orders of magnitude. Even for moderate droplet diameters of 200µm and spacing of 500µm, densities of 400 spots per cm^2 can be reached. This enables the deposition of thousands of analytes on a standard microscope slide. Several techniques to create these arrays have been presented so far. Most companies targeting the low-price market for low- and medium-density biochips (up to 1000 analytes) use either contact printing or ink-jet-like methods for placing the droplets. On the high-end side, Affymetrix has patented a mask-supported photochemical on-chip synthesis to produce ultrahigh-density microarrays. A big challenge for all is to make microarray technology reproducible. Crucial problems are associated with the reliability of signals which are rooted in: biochemistry (e.g. attachment of fluorescent dye to analytes), droplet generation (variations or failures of spots) and detection (large background noise, low signal level).On the other hand, a prerequisite for the success of microarray technology on an industrial level is without doubt the ability to produce large volumes of cheap biochips. In our view, none of the presently available array writing devices meets all industrial requirements on costs, speed and reliability at the same time. With the development of TopSpot at HSG-IMIT and IMTEK for our industrial partner BioChip Technologies (Freiburg, Germany), we fulfilled these challenging demands. A robust and scalable printhead that can be integrated in various microarray writers was supplied. A manually operated workstation up to a fully-automated industrial production plant producing slides each carrying thousand analyte spots with a throughput of up to 5000 slides per day can be constructed around this printhead.

Outline of the TopSpot Principle

The TopSpot print head (Fig. 1) consists of a central silicon wafer sandwiched between two Pyrex wafers. The silicon wafer is structured by dry-etching and then stacked by anodic bonding to the outer Pyrex wafers. A smaller and a larger window on the lower and upper side are left uncovered. In a central array, the nozzles are etched through the full wafer depth. Each of the nozzle tips is structured from the backside for optimised droplet release.

In the 96-nozzle layout the interface to the macro world is realized by the fluid reservoirs (Ø 1.5mm) aligned in two pairs of 24-reservoir rows on the outer part of the chip. Their 2.25mm-spacing is chosen according to the standard 1536-well microtiter plate format. With appropriate automation, all reservoirs may be filled in two pipetting cycles. The TopSpot module features a so-far unmatched parallelism on the input as well as on the output side, which is crucial for the high-speed production of biochips.

The main pathway of the fluidic conduits between reservoirs and nozzles is situated on the bottom side of the chip which is sealed by the 150µm Pyrex wafer. Each of these channels is connected by two through holes at its ends to the upper side of the chip (Fig. 1). One end directly accesses the reservoir. The other through hole connects to a short channel eventually conducting the fluid to the nozzle. The liquid is moved by mere capillary forces. In order to increase the capacity of fluid storage to about 5µl in the tightly spaced 96-nozzle layout, the silicon reservoirs are furthermore extended by holes in the upper 3mm-thick Pyrex wafer. The reservoirs are covered by a plate which can be cooled by a Peltier element to prevent evaporation.

For actuation, a PEEK stamp is used. A gap of roughly 300µm is left between the bottom of the stamp and the nozzle array. The stamp is pushed towards the print head by a piezo actuator providing very fast, precise and reproducible control of the whole actuation process. Upon actuation, the stamp moves by about 50µm. In this way, a comparatively large compression ratio DV/V can be reached, guaranteeing a high amplitude of the pressure signal. Due to the principle of isotropic pressure, the same pressure ramp is applied to all nozzles driving an array of droplets out of the bottom side of the chip. In order to compensate the pressure gradient after printing, a tiny through hole is drilled across the stamp (Fig. 1).

stabilize after the first droplet array. Figure 2 displays a 2ms sequence of the droplet ejection process. It can be observed that after actuation the droplets break off cleanly from nozzle and no satellites are formed.

The reliability and uniformity of droplet ejection is very high. No dogging or sudden failure during print cycles was observed. Droplet diameters in the liquid phase primarily depend on the nozzle diameter. For a series of 1400 consecutive prints of DI water, the mean droplet volume was 1.41nl. The standard deviation of 0.04nl falls below 3%, which is quite outstanding for volumes in this range.

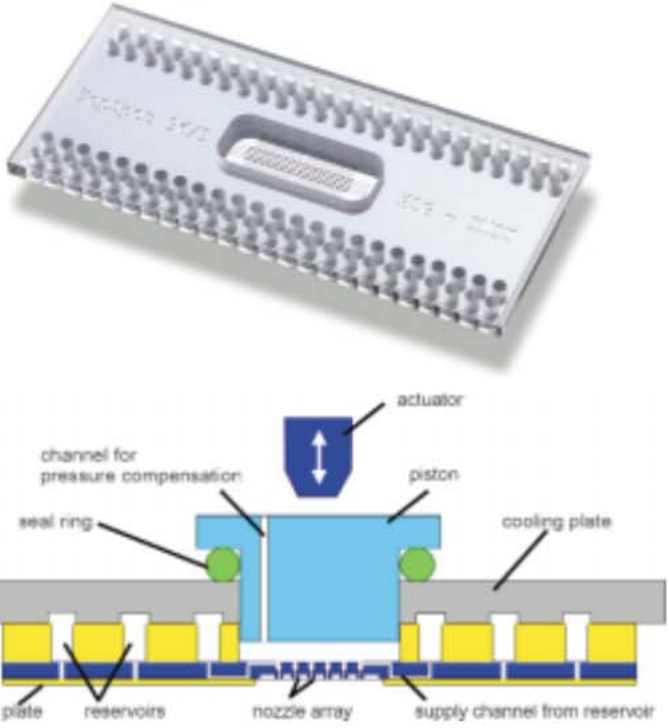

Fig. 1: Picture and schematic of the 96-nozzle TopSpot print head and its actuation.

It is important to stress that TopSpot technology does not address single nozzles. On the contrary; all nozzles see the same pressure ramp leading to the simultaneous ejection of all droplets. For Biochip production, this is no limitation at all.

Results

We have measured the quality and reliability of our 24-nozzle TopSpot print heads. Putting the print head into operation proves to be quite uncomplicated. After filling the reservoirs, printing results already

Commercial Implementation

In the rapidly evolving biochip business, there is a strong request for reliable micro-arraying machines. On one hand, many laboratories are still in an explorative stage of their proprietary biochip technology. For their purposes, compact, moderately prized micro-arrayers for the low-volume production of low- and medium-density biochips are demanded. This market segment is addressed by our manually operated "TopSpot Entry Arrayer" (TopSpot /E) and the partially automated "TopSpot

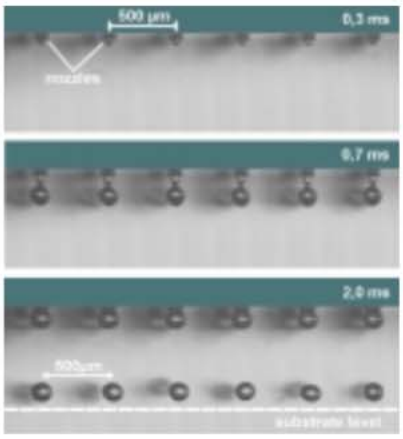

Fig. 2: Droplet formation sequence. Droplets ejected without satellite formation in a 2ms time interval onto the substrate situated at 500µm from the print head.

Modular Arrayer" (TopSpot /M). The TopSpot/M features an extendible amount of print heads optionally completed with a contact and ink-jet printers which are already commercially available.

On the other hand, some companies possess ready-to-go solutions requiring the high-throughput production of low-cost biochips. To our knowledge, no product is currently being offered to serve this rapidly expanding market segment. Our fully automated "TopSpot Production System" envisions this interesting market.

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