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TopSpot Quality
MICROARRAYS

TopSpot technology introduces a new concept into the microarray laboratory: highly parallel non-contact dispensing of bioanalytical reagents for the production of low to medium density microarrays.

The technology is based on micromachined multi-channel printheads combined with a piezo-driven droplet ejection process. Different automation environments for these print modules allow TopSpot technology to cover needs from decentralized laboratories to core facilities.

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Introduction

Microarray technology has revolutionized pharmaceutical research and diagnostics. It has opened new perspectives for gene expression analysis, pharmacogenomics and proteomics [1]. Today, many routine applications of microarray chip technology exist, which are focusing on dedicated sets of genes or proteins of interest, rather than a large number of different analytes. One of the main challenges for the commercial success of these low to medium density microarrays is a fast and reliable method of production. Currently the most commonly used technology to produce DNA microarrays is the contact-based pin printing technology. The main drawback of this technology is the reloading of the needles and washing steps in-between, considerably slowing down the whole print process. Another limitation in using needles is the occurrence of carry-over at the arrayed spots. For protein microarrays the variation of the deposition properties of different samples onto glass slides has turned out to be critical for the quality of the microarray. As the biochemistry of proteins is orders of magnitude more complex than DNA biochemistry the influence of the metallic needle on protein structure is not clear yet [2].

Overcoming the limitations – with TopSpot technology

With TopSpot technology, invented by the HSG-IMIT (Hahn-Schickard-Gesellschaft) in cooperation with Imtek at the University of Freiburg, a reliable and robust method exists for highly parallel printing of microarrays. The use of this non-contact spotting method in comparison to traditional pin-printing makes it possible to raise the throughput in the production of low and medium density microarrays by almost two orders of magnitude. Other advantages are the prevention of cross-contamination and the high reproducibility of the printing process.

Tab. 1: Used printing buffer solutions.

Printing media	Highest validated concentration	Recommended concentration
nucleic acids		
Oligoncleotides 20mer, 40mer, 60mer	100 µM	20 µM
cDNA	200 µg/ml	200 µg/ml
Salmon sperm DNA	500 µg/ml	150 µg/ml
BACs	200 µg/ml	200 µg/ml
proteins		
BSA (Dy630 labeled)	1,500 µg/ml	200 µg/ml
various antibodies (Dy630 labeled)	400 µg/ml	200 µg/ml
Lysozym	200 µg/ml	200 µg/ml
DNA K	2,000 µg/ml	200 µg/ml
ß-Galactosidase	100 µg/ml	100 µg/ml
Human Serum	0.25x	0,25x
carbohydrates		
Aminodextran	2,000 µg/ml	not tested
Optodextran	250 µg/ml	not tested
Saccharose	250 µg/ml	not tested
components to mediate coupling		
EDC (N-Ethyl-N'-(3-dimethylaminopropyl) caroimide)	120 µg/ml	120 µg/ml
NHS (N-Hydroxysuccimide)	20 µg/ml	20 µg/ml

The core of the TopSpot principle is a microfabricated silicon/glass printhead [3]. This printhead facilitates the simultaneous dosing of many different liquids at a 500 µm grid (Fig. 1). The number of droplets dispensed depends on the type of printhead used. Printheads with 24 and 96 reservoirs, respectively have been realized and operated. Since the reservoirs are arranged in standard microtiter plate format, filling of the printhead with up to 96 different print samples can be done manually or by using liquid handling robots. Liquid is drawn from each reservoir to the corresponding nozzle automatically by capillary forces. The ejection of sample droplets from the printhead is mediated by a piezostack actuator, which drives a piston into the air chamber of the printhead. This generates a pressure pulse that affects all nozzles simultaneously and accelerates droplets out of the nozzles (Fig. 2). The liquid in the reservoirs is usually sufficient for 1,000 to several thousand prints. Due to the economical design of the fluidic channels, the amount of expensive sample lost through dead volume is close to zero (< 0.4 µl). The integrated format conversion in the printhead is one of the key factors for increasing the printing speed. In contrast to the pin-printing technology, a contact-free high-throughput production is possible, which eliminates problems arising from the varying adhesion forces between probes, needles and the substrate surface.

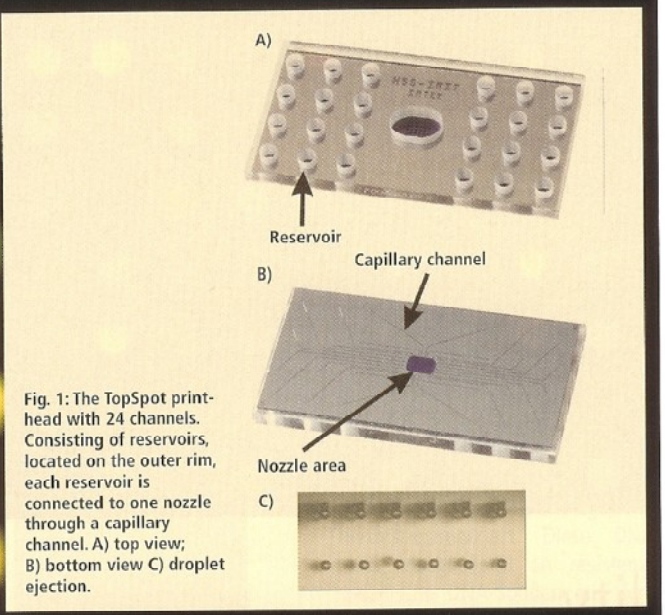

Fig. 1: The TopSpot printhead with 24 channels. Consisting of reservoirs, located on the outer rim, each reservoir is connected to one nozzle through a capillary channel. A) top view; B) bottom view C) droplet ejection.

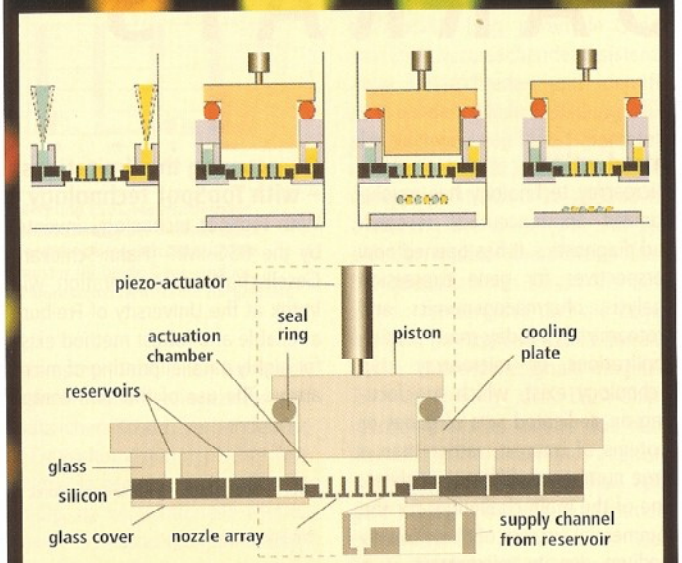

Fig. 2: Piezo-mediated droplet dispense. The TopSpot printmodule with the printhead in place; A cross-section, B experimental set-up. Movement of the piston causes a compression of the air in the air chamber, this pressure ultimately causes droplets to be ejected from the nozzles.

TopSpot technology in action

Printing microarrays comprises a large field of applications and requires flexible printer systems. Different applications need different buffer systems, depending on printing media, coupling chemistry or surface properties of slide substrates. Many applications have been established and evaluated for the TopSpot technology, including oligonucleotides, cDNA, plasmids, standard antibodies, antigens, DNA-protein complexes, and cells. Besides dispensing standard buffers and solvents, TopSpot technology has proven to reliably print DMSO and up to 40 % v/v of glycerol [3] (Tab. 1). To guide TopSpot users 'standard operating procedures' (SOP's) are established for a multitude of probe types in combination with medium composition and a host of substrates. Extensive experiments showed that the volume of dispensed droplets is only slightly depending on characteristics of printing fluid, mainly viscosity and surface tension. In general the reproducibility of droplet volumes of about 1 nl within one single channel was better than 1 % independent from used printing buffer solution (Tab. 1). Further experiments comprising thousands of printed arrays ascertain cross-contamination and satellite-free printing of microarrays. In contrast to reloading pin-printing techniques a prime critical point of microarray production is solved, leading to high quality whilst high-throughput microarray fabrication.

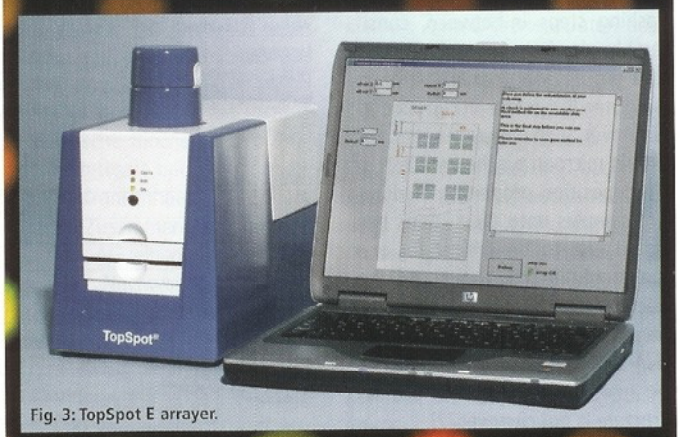

Fig. 3: TopSpot E arrayer.

The new TopSpot E arrayer

TopSpot technology is available in different scales in order to meet the specific requirements imposed by applications and throughput. So far, instruments comprising one up to fifteen print modules are operating in laboratories throughout Europe. All instruments employing TopSpot technology have in common, that they require minimal training and setup time. The new TopSpot E microarray printer (Fig. 3) – offered by Geneworx AG in Oberhaching – contains one single print module and has been specifically designed for low to medium throughput demands. Depending on the printhead used, it typically produces arrays containing 24 or 96 spots, respectively, with a typical spot diameter of about 200 µm. With TopSpot E, a personal microarray spotter is now available, which combines the advantages of non-contact printing technology with its ease of use, small size and low cost, making it the ideal choice for decentralized laboratories.

References

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