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TECHNICAL NOTE

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Highly parallel dispensing of chemical and biological reagents

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Abstract We present a technology for the highly parallel dispensing of a multitude of reagents. It allows one to dispense up to 96 different reagents simultaneously in a fixed array, in a volume range of 100 pL up to several nL. The pitch of the dispensed droplets can be as small as 500 µm. All channels are fired simultaneously, giving an unprecedented throughput. The system was originally developed for the high-throughput fabrication of microarrays, but can easily be adopted for other applications such as highly parallel filling of nanotiterplates. Based on our standard configuration we achieved droplets with 125-µm in-flight diameter (1.2 nL) with a CV of <1%.

Keywords Microarrays · Biochips · Dispenser · Nanotiter plates · Microfluidics · Non-contact printing

Introduction

Microarrays are a collection of miniaturised test sites with biomolecules that are arranged on a solid substrate. This permits many tests to be performed at the same time to achieve higher throughput and speed. The fast growing number of applications shows the extraordinary surge of interest in microarray technology [1, 2]. The biomolecules used range from oligonucleotides, cDNA and plasmids to complex molecules like proteins (e.g. antibodies) [3, 4, 5]. One of the main challenges for the commercial success of microarrays is a fast and reliable method of production. There are two basic strategies for making microarrays with oligonucleotides: the on-chip synthesis (in situ) or the external synthesis of the biomolecules followed by dispensing onto the substrate by contact or non-contact printing systems. For the growing field of protein applications where on-chip synthesis is not possible, non-contact printing seems to be the most feasible technique [6]. A limitation for microarray printing techniques is the format conversion between the reservoir pitch of commonly used storage plates (i.e. microtiter plates) and the microarray format. Mainly a complex automation concept is used to handle this. The TopSpot technology [7, 8, 9], as it is used in this publication, uses a different concept and through this we can arrive at a far higher throughput compared to other technologies.

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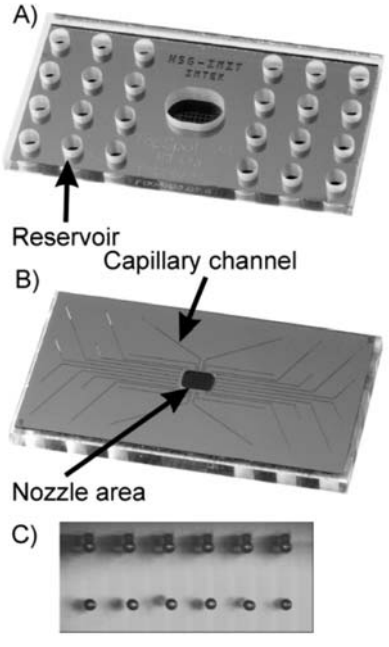

Fig. 1A–C The TopSpot printhead with 24 channels consisting of reservoirs, located on the outer rim. Each reservoir is connected to one nozzle through a capillary channel: **A** top view, **B** bottom view, **C** ejected droplet array

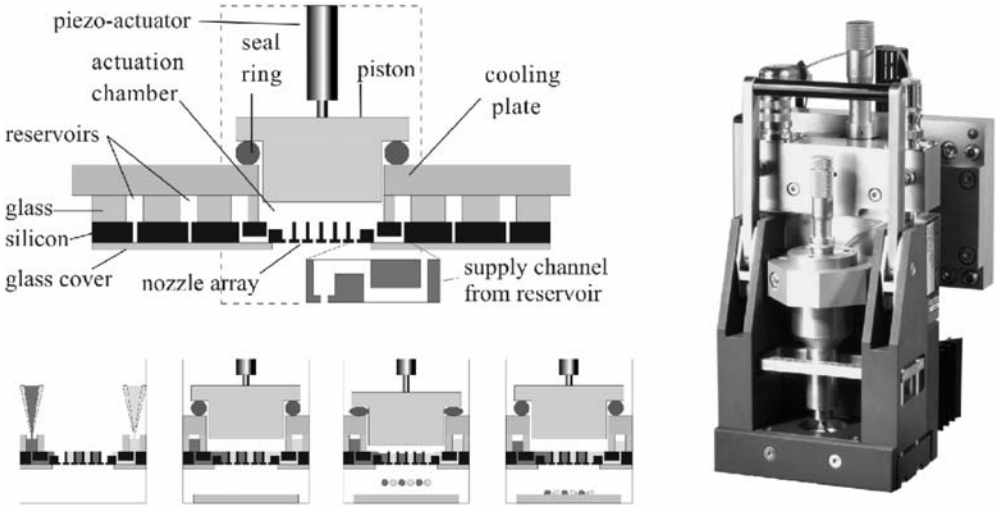

Fig. 2A, B The TopSpot print module with the printhead in place; **A** cross-section, **B** experimental set-up. Movement of the piston causes a compression of the air in the air chamber; this pressure ultimately causes droplets to be ejected from the nozzles

Table 1 Test media

Name	Description	Supplier	Remark
SSC (saline sodium citrate)	20×SSC=3 M sodiumn chloride (NaCl); 0.3 M sodium citrate	Invitrogen	pH 7
PBS (phosphate buffered saline)	10×PBS=1.36 M sodiumn chloride (NaCl); 0.02682 M potassium chloride (KCl); 0.05 M disodium hydrogenphosphate (Na_2HPO_4); 0.017635 M potassium dihydrogenphosphate (KH_2PO_4)	Gibco BRL	
DMSO	dimethyl sulfoxide	Merck	viscostiy 2.14 mPas
Glycerol		Arcos Organics	viscosity 1,000 mPas
Oligonucliotide	20-, 40- and 60-mers with Cy3 label	Genescan AG	
Antibodies	donkey anti goat IgG with Cy3 label	Dianova (Jackson)	
Cy3, Cy5	cyanine Dye 3 resp 5, fluorescence dye	Amersham	

Materials and methods

The TopSpot principle

The TopSpot technology uses printheads which, very much like inkjet printers, consist of bulk reservoirs of similar size and exactly the distances of microtiter plates. These reservoirs are connected, through capillary channels, with a nozzle array (Fig. 1). The arrangement is such that there is a one-to-one relationship between the placing of the reservoirs and nozzles. Distances between the nozzles correspond to the desired distances between the spots on the microarray. So far printheads with 24 and 96 channels have been made, each with a nozzle pitch of 0.5 mm and a reservoir pitch of 4.5 and 2.25 mm, respectively.

Each reservoir is filled with a different bulk reagent (several µL). The reagents are transported automatically through capillary forces from the reservoirs to the nozzles. These forces also facilitate the refilling of the nozzles after actuation. The print head is placed in a so-called print module. In this print module a piston is positioned above the filled nozzles, defining an air chamber (Fig. 2). The piston is actuated by the piezo-actuator causing a compression of the air in the chamber. This pressure pulse is transferred into the liquid causing the complete array of nozzles to eject one droplet of typically 1 nL each.

Materials

Experiments were performed with standard TopSpot equipment (HSG-IMIT) as described above. To test the different application fields, printing was done with pure buffer, buffer with oligonucleotides, buffer with antibodies and subsequently a complete assays was performed. Table 1 lists all media used with their composition and supplier.

The oligonucleotides used were Cy3-labelled for direct verification. First we measured the ejected droplet size through stroboscopic observation. Secondly we spotted onto silanised slide surfaces (Arraylink hydrophob, Genescan AG) and analysed them by using a fluorescence reader (BioAnalyzer, LaVision Biotech). In this way we gained insight in the quality of the fabricated microarrays.

Results

Results of stroboscopic observation are shown in Fig. 3 (3xSSC buffer). The volume was determined to be 1.2 nL with a measured coefficient of variation (CV) of below 1%. CV is taken from the measurement of 250 ejections from one nozzle. Further results for a number of relevant buffer sys-

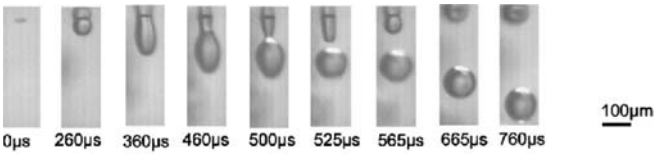

Fig. 3 Stroboscopic observation of droplets (3xSSC buffer). Droplet size of 125 µm with a CV below 1%

Table 2 Relevant buffers and the generated droplet volume at the same actuation settings

PBS			SSC		
Conc.	Droplet volume (nL)	CV (%)	Conc.	Droplet volume (nL)	CV (%)
0.25×	1.121	0.32	0.25×	1.096	0.20
0.5×	1.121	0.22	0.5×	1.102	0.29
1.0×	1.200	0.33	1.0×	1.119	0.19
1.5×	1.265	0.29	1.5×	1.136	0.16
2.0×	1.310	0.13	2.0×	1.188	0.24
3.0×	1.289	0.17	3.0×	1.207	0.24
DMSO			Glycerol		
5%	1.28	0.19	5%	1.53	0.08
10%	1.104	0.10	10%	1.204	0.33
20%	1.219	0.24	20%	1.419	0.32
30%	1.339	0.29	30%	1.478	0.55
40%	1.488	0.27	40%	1.541	0.33
50%	1.550	0.25	50%	1.518	0.30
3×SSC+*x*M betaine			3×SSC+x%Nonidet		
0.2 M	1.130	0.22	0.0005%	1.193	0.22
0.3 M	1.089	0.26	0.001%	1.318	0.10
0.4 M	1.093	0.22	0.01%	1.359	0.48
0.5 M	1.120	0.14	0.05%	1.304	0.32
1.0 M	1.268	0.33	0.1%	1.243	0.39
1.5 M	1.427	0.22	0.5%	0.97	0.81

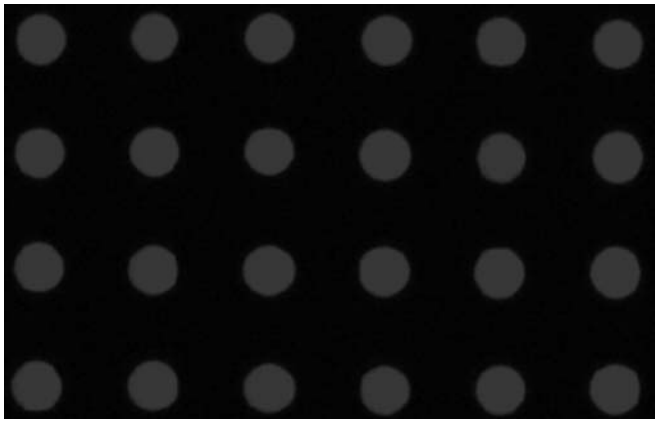

Fig. 4 Fluorescent image of 1 µM Cy3-labelled 20-mer oligonucleotide spotted in SSC buffer. Spot size 209 µm, CV<2%; integral intensity of the complete array has a CV<5%, for all 24 spots

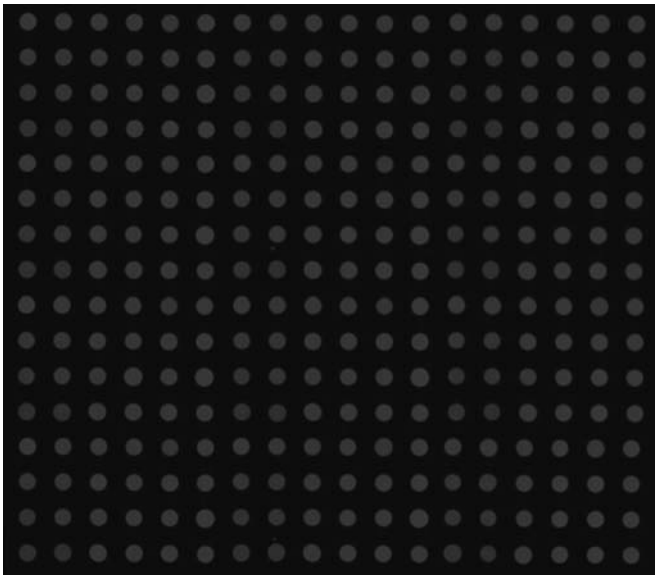

Fig. 5 4×3 arrays with Cy3-labelled Donkey anti goat IgG (0.66 mg L) in 1xPBS on ArrayLink hyphob. Coefficient of variation of the signal intensity below 4%

tems are shown in Table 2. The viscosities of the evaluated media varied from values similar to that of water (1 mPas) up to 30% (v/v) glycerol/water mixture (1.9 mPas). Surface tensions were not measured but did change considerably as well. For these measurements the print head was actuated with a frequency of 1 Hz, thereby also showing the possible speed of production.

Biologically relevant buffers were also spotted with Cy3-labelled 20-mer, 40-mer and 60-mer oligonucleotides in concentrations of 1 µM on silanised hydrophobic slide surfaces. The resulting spots were analysed using the fluorescence reader. A typical result is shown in Fig. 4, more results are summarised in Table 2. The spot diameter was measured to be 209 µm with a CV below 2 %. The CV for the integral fluorescence intensity of the 24 spots within one array was below 5%. In Fig. 5 an example of a protein array with Cy3-labelled donkey anti goat IgG is shown. The integral signal intensity of the fluorescence signal had a maximum standard deviation of 4%. Figure 6 shows the possibilities of doing complete assays on a microscale. Shown is an array of spotted antibodies after incubation with a mixture of their respective antigens.

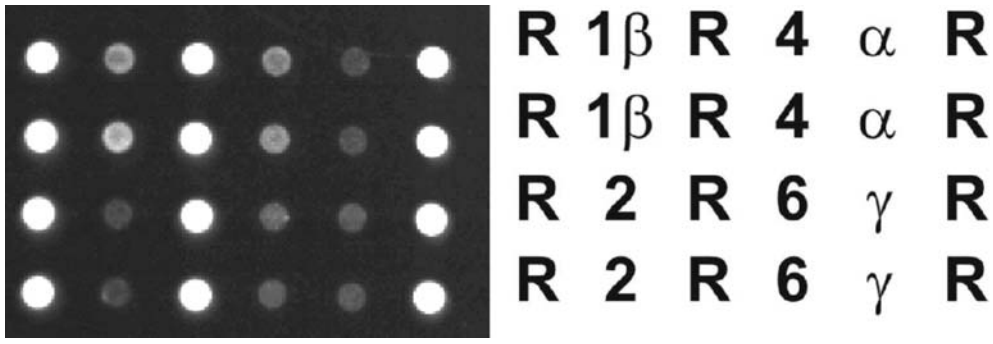

Fig. 6 Array of antibody antigen assays; spotted were (see chip layout; *right panel*) β anti-ILβ (Interleukine), *2* anti-IL-2, *4* anti-IL-4, *6* anti-IL-6, α anti-TNFα (tumor necrosis factor), γ anti-IFNγ (interferone), *R* BSA directly labelled with Cy5 as reference (bovine serum albumine), all in 6 nM concentration). *Left panel* scanner image after incubation with a mixture of corresponding, labelled (Cy5), cytokines (courtesy of Zeptosens)

Conclusions

A spotting system with very good spotting quality and an unprecedented speed of production is reported here. The typical CV of ejected volume was between 1% and 4% for the shown examples. Ejection was possible with a speed of 1 Hz, meaning that a complete array consisting of 24 spots can be printed each second. Several applications have successfully been demonstrated. Two core factors contribute to high speed fabrication: integrated format change, which allows for a narrow pitch between the dispensing nozzles, and the high number of parallel channels that spot at the same time. The accuracy is very high due to the use of photolithographic techniques for the definition of the print head geometry.

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