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Impact of medium properties on droplet release in a highly parallel nanoliter dispenser

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Abstract

We report about the correlation between satellite free droplet release and liquid viscosity in a highly parallel, pressure driven nanoliter dispenser. In extensive studies we found that for liquids of different viscosities the duration of the pressure pulse is the predominant effect compared to pressure amplitude. This result is of essential importance when actuation parameters have to be adopted for different media like oligonucleotides, DNA or protein solutions as it is the case for the non contact high throughput fabrication of microarrays [J. Ducrée, H. Gruhler, N. Hey, M. Mueller, S. Békési, M. Freygang, H. Sandmaier, R. Zengerle, TopSpot—A new method for the fabrication of microarrays, Technical Digest, in: Proceedings of the Thirteenth IEEE Annual International Conference on Micro Electro Mechanical Systems, Mizyazaki, Japan, 23–27 January 2000, pp. 317–322]. For oligonucleotides printing we found coefficient of variations (CVs) of droplet diameter to be better than 1% within one single dispensing channel and 1.5% within all 24 channels for each used printing buffer. The CVs for protein printing were slightly higher but likewise for the oligonucleotides below 1%. Experiments with oligonucleotides as well as with different proteins showed ascertained carry-over and cross-talk free printing of DNA and protein microarrays. With it a prime critical point of microarray production is solved, leading to high quality whilst high throughput microarray fabrication.
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Keywords: Microfluidics; Droplet dispensing; Highly parallel; Microarrays; Oligonucleotides; Proteins

1. Introduction

High throughput screening methods are used in many domains of life sciences as well as in pharmaceutical and chemical sciences. Miniaturization, automation and parallelization allow to decrease costs of often expensive materials and leads to faster analyzing times [2]. The microarray technology has revolutionized the fields of biotechnology and life sciences. It has opened new perspectives for decoding the human genome, for molecular gene and DNA diagnostics, proteomics as well as for new ways in pharmacogenetics. The DNA microarray technology is established in many fields of applications [3]. But as the biochemistry of proteins is orders of magnitude more complex than DNA biochemistry the protein microarray technology is in the majority of cases only in a laboratory stage [4]. Furthermore there are already efforts in binding complete cells to substrates.

The commonly most used technology to produce microarrays is the contact based pin printing technology. But one main drawback of the pin printing technology is the time consuming reloading of the pins and washing steps in-between, so that pin printing has a limitation in speed, even with use of 16 or more pins in parallel. Another big problem of using pins is carry-over of the arrayed spots and especially in the field of protein microarrays the variation of the deposition properties of protein probes onto glass slides, which is leading to inhomogeneous amounts of probes on the microarray. Also the influence of the metallic needle on protein structure is unclear. The TopSpot technology overcomes these problems and enables high throughput printing of microarrays in advanced quality [1].

1.1. Principle of operation

The TopSpot technology was developed and optimized for high throughput printing of DNA oligonucleotides. This new technology considerably accelerates the application of substances on a substrate, mainly modified glass or plastic

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slides, and permits mass production of microarrays, often also called biochips, at reasonable costs.

The technology is based on a micromachined printhead, the fabrication of this TopSpot printhead is described in [1]. The printhead formats allow the simultaneous application of 24 and 96 different probes in one step, respectively. The TopSpot printhead consists of three layers, pyrex glass, silicon and another pyrex layer. The probes are contained in reservoirs drilled in the upper pyrex glass wafer and can be filled by standard liquid handling robots. The glass wafer is bonded to the micromachined silicon wafer by anodic bonding, so that a covalent binding of glass and silicon wafer is achieved. The reverse side of the silicon wafer is bonded to a thin pyrex glass wafer with a square opening for the outlet nozzles. A recess in the upper Pyrex (actuation chamber) opens the nozzles to the upper side of the printhead. Every reservoir is connected with one nozzle in the central area of the print head (nozzle array) via an etched microchannel system in the silicon wafer. Liquid is drawn to the nozzles simply by capillary forces. The actuation of the printhead is done by a piezostack actuator as depicted in Fig. 1. The actuator drives a piston into the actuation chamber of the micromachined printhead. This generates a pressure pulse that affects all nozzles simultaneously. If the pressure pulse is high enough, it overcomes the capillary forces of the nozzles and the surface tension of the fluids and droplets are accelerated out of the nozzles. To achieve a homogenous ejection of the droplets, the nozzle array is coated with hydrophobic silane [5].

One of the major advantages of the TopSpot technology over other nanoliter dispensing systems (e.g. inkjet printer) is that every nozzle can be supplied with another printing medium at the same time. So in contrast to the present proceedings (e.g. pin printer, piezo tips) a contact-free high throughput production is possible. This eliminates problems arising from the varying adhesion forces between probes, pins and the substrate surface.

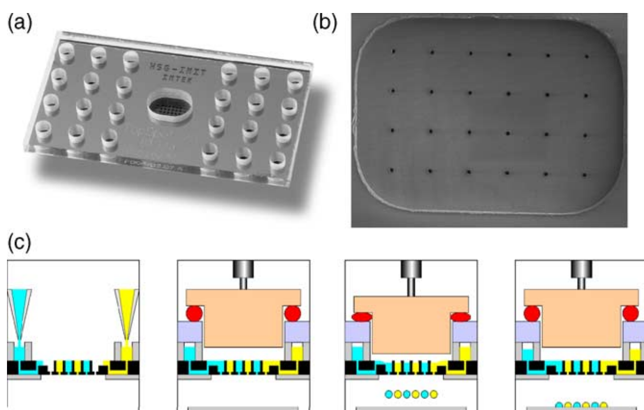

Fig. 1. (a) Picture of a printhead; (b) SEM picture of the 24 nozzles on the bottom side of the printhead; (c) working principle of TopSpot. (1) Filling of the printhead reservoirs; (2) placing a piston into the printhead; (3) actuation by moving of the piston; (4) retraction of the piston.

Printing microarrays requires very flexible printer systems. Different applications need different buffer systems, depending on printing media, coupling chemistry of used microarray slides or surface properties. This publication reports on the evaluation of operating range and limits of the pressure driven droplet release and on the correlation between specific properties of printed media and corresponding printing conditions.

2. Experimental

2.1. Experimental setup

In our publication we investigated printing with 24 channel printheads. Parameters for diameter and pitch of the circular nozzles were 50 and 500 μm. To assure the non-existence of sample cross-contamination in the printhead we made extensive cross-talk tests. Therefore every second reservoir was filled with 1 μM Cy3-labeled oligonucleotides and the other reservoirs with pure printing buffer. The reagents have been printed onto microarray slides and detected with a fluorescence reader (La Vision Biotech, Germany, sensitivity up to <0.8 Cy3 molecules/μm^2). Although without cross talk they look like a checker board. The same tests were also done with a variety of proteins, such as Cy5-labeled BSA or antibodies (each 200 μg/ml).

Since the printhead is reused it is important to evaluate the washing procedure of the printhead to guaranty carry-over free usage of the printhead with following fillings. Therefore the checkerboard-like filled printhead of the cross-talk test was washed in an ultrasonic bath with 5% (v/v) RBS N and afterwards rinsed in excess of deionized water (standard washing procedure). Then the checkerboard-like filling was inversed, so that every nozzle, which was filled with Cy3-labeled oligonucleotides is now filled with buffer solution. In the fluorescence reader, no leavings of former filling should be detectable, even at very high exposure times. The carry-over test was also done with Cy5-labeled proteins in different printing buffers, showing that the standard washing procedure was inadequate for proteins. So the standard washing procedure was adapted. Before the ultrasonic bath an additional rinsing step with 5% (v/v) RBS N and after the rinsing in excess of deionized water a second ultrasonic bath with deionized water was introduced.

Surface tension and viscosity of printing media are the properties with the major impact on the droplet dispensing process. The main portion of the printing medium is the printing buffer. In the field of microarrays very different printing buffers are reported in publications. So we investigated a wide range of different buffers and additives in different concentrations a wide range of viscosities and surface tensions (Fig. 5). In all cases we determined the optimum conditions for a stable and satellite free droplet ejection process. The surface tension of the different printing buffers were measured by a drop shape analysis system

(DSA10 Mk2, Kruess, Germany) according to the "pendant drop" method [6]. For the correlation between printing media and needed printing conditions the piston movement of the printer device was recorded during printing process using the laser–doppler–vibrometer (OFV 1102, Polytek, Germany). The actuation pressure pulse was calculated from the piston movement and the actuation chamber volume. These data enable us to analyze the correlation between amplitude and duration of the pressure pulse, on the one hand, and the quality of droplet release, on the other hand.

A stroboscopic camera (Visit Video Stroboskop MOCRON-RT, Germany) was used to evaluate droplet dispensing and droplet flight of different printing media towards the microarray slide in real time. The determination of droplet volume by gravimetrical measurements using a high precision balance is difficult due to volumina in nanoliter range and evaporation during measurement. So to avoid evaporation the measurement should be made in a humidity chamber in which the evaporation is in balance with water absorption of the droplets, but this is very difficult. Using the fact that droplets form a nearly spherical shape due to the surface tension we circumvent these problems by using a stroboscopic camera and capture the dispensed droplets during the whole dispensing process. The trigger signal of the camera was adapted to the trigger signal of the piezostack actuator to get a time resolved measurement of the droplet dispensing process. As a precondition to use this measurement system reproducibility of the sequential events is essential. For a more precise analysis of the recorded droplet volume during flight and the droplet impact onto the microarray substrate the image processing system NeuroCheck® (NeuroCheck®, Germany). was used. A programmed measurement process was developed to measure the diameter of dispensed spots to ensure the correct evaluation of the spots. In our case the spot volume can be determined by geometric outline analysis of the dispensed spots. Considering the resolution of used camera at maximum magnification we assume a measurement error of ± 1 pixel. Due to the limited resolution of the used camera and the arrangement of the nozzles on the printhead it is not possible to evaluate all 24 nozzles at the same time by this stroboscopic method.

To observe all 24 droplets in one step we used a fixed camera system, mounted on the TopSpot printer device. By using a mobile axis system as slide carrier it was possible to evaluate the droplet flight and impact onto microarray slide. Also each printed 24 spot array on slide was photographed automatically right after the print. Now NeuroCheck® was not only used for determination of spot diameter but also for evaluation of horizontal deflection of the spots from their correct 500 μm spacing. Correct size and position of spots is essential for microarray usage. Therefore this system is able to do the quality control of a microarray fabrication. To show that higher densities are achievable the spacing between the spots was lowered by printing between the spots of already printed arrays, resulting in a 250 μm spacing of spots.

As an alternative method to the camera systems a typical microarray approach was used by mixing the printing buffer with Cy3-labeled oligonucleotides (1 μM final print concentration) and for the protein microarray application with Cy5-labeled BSA or Cy5-labeled antibodies (200 μg/ml). Now the fluorescence signal can be used as a measure of dispensed volume. By using the fluorescence reader we determined the intensity of fluorescence signal of dispensed droplets on the microarray slides. The limits of the TopSpot printheads according to viscosity and surface tension of printing media were tested by using different amplitudes and durations of the pressure pulse to dispense different concentrations of glycerol–water and surfactant-buffer solutions. The printhead was filled with one concentration of a solution and arrays were printed. The highest stable (over 250 arrays) printable concentration was determined.

The following chemicals and materials were used for the experiments of this publication.

- 10× phosphate buffered saline (PBS), Gibco BRL.
- 20× saline sodium citrate (SSC), Gibco BRL.
- ArrayLink buffer, Genescan Europe AG.
- Boric acid, Sigma–Aldrich.
- Sodiumcarbonate, Sigma–Aldrich.
- Sodiumbicarbonate, Sigma–Aldrich
- Dimethylsulfoxid (DMSO) [$(CH_3)_2SO$], Merk.
- Glycerol 99% [$C_3H_8O_8$], ACROS ORGANICS.
- Betaine monohydrate [$C_5H_{11}NO_2 x H_2O$], Fluka.
- Nonidet® P40 Substitute, ionic surfactant, Fluka.
- RBS N, non-ionic surfactant pH 7, ROTH.
- Oligonucleotides:Cy3 20mer (5′-5ac gta cgt acg tac gta cg-3′), BIG-Biotech GmbH, Germany.
- Bovine serum albumine (BSA), fraction V, VWR.
- Donkey anti rabbit IgG-Cy5 (H + L), Dianova.
- Donkey anti mouse IgG-Cy5 (H + L), Dianova.
- Donkey anti goat IgG-Cy5 (H + L), Dianova
- ArrayLink hyphob, epoxy-modified glass slides, Genescan Europe AG.

3. Results and discussion

Extensive experiments using labeled oligonucleotides and different labeled proteins showed the total isolation of the different microchannels, nozzles and dispensed droplets without any cross talk even after thousands of printed arrays. For the re-usage of printheads the experiments showed that even after washing and re-using of printheads no carry-over of formerly printed media was detectable (Fig. 2). For the protein printing the washing procedure of the printheads was improved. With it we ensured cross-contamination free and reliable printing of many proteins (Fig. 2). The improvement was necessary due to fact that compared to the a simple poly-anion DNA the biochemistry of proteins are in orders of magnitude more complex and very diverse. So the molecules in many proteins have in general tendency to stick

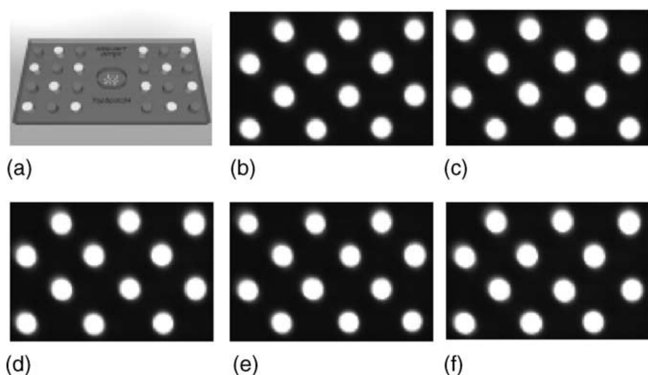

Fig. 2. Carry-over test: the printhead was filled with Cy5-labeled BSA and buffer like a checker board (a). The printed microarrays were evaluated in a fluorescence reader (b). Afterwards the printheads were washed, refilled with the inversed checkerboard filling and printed (c). The whole procedure was repeated five times (b–f). In the quantification of the 4×6 arrays only the spots with Cy5-BSA are visible, that means both no cross-contamination and carry-over was detectable using the fluorescence reader.

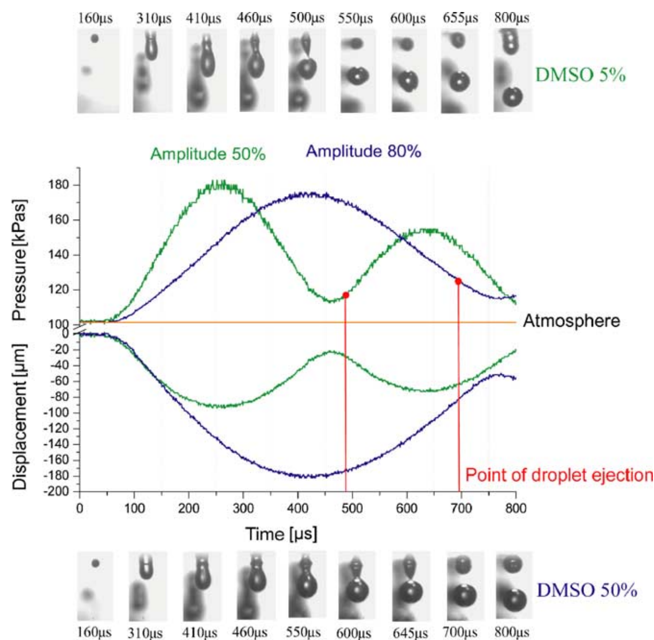

Fig. 3. On the top and the bottom of the graph the droplet ejection of two different concentrations of DMSO is shown (grey lines: DMSO 5%, dark lines: DMSO 50%). The different used amplitudes of the piezo stack actuator are indicated in blue and green based on possible maximum deflection. In the graph the displacement of the of the piston (lower curves) and the calculated pressure pulse (upper curves) are plotted against the time. The points of droplet release are indicated.

to the hydrophobic coating of the printhead nozzles than oligonucleotides. The improved standard washing procedure is more stringent so it fulfils the requirements of DNA and protein usage and cleans the printhead properly. The experiments ascertain carry-over and cross-talk free printing of DNA and protein-microarrays and in contrast to reloading pin printing techniques we have solved for our system a prime critical point of microarray production, leading to high quality whilst high throughput microarray fabrication.

Optimal droplet dispensing conditions are stable over hundreds of dispensing cycles and satellite-free. Generally droplet release took place in the first minimum of pressure pulse (Fig. 3). The oscillation of pressure pulse is due to the fact that the piezostack actuator oscillates around the desired stroke (data not shown). The withdraw of piston generates a negative pressure gradient and facilitate thereby the droplet release. We correlated the amplitude and duration of the pressure pulse under optimal droplet dispensing conditions of a specific printing solution to the liquid properties of used buffer. So we found that the droplet release takes longer for higher viscous fluids (Fig. 3). This can be explained with the higher friction of higher viscous fluids. So the response of the fluid takes more time and the pressure pulse has to take longer. It seems not necessary to charge higher pressure amplitude for higher viscous fluids. The highest stable printable viscosity and lowest surface tension were 30 vol.% glycerol–water and 0.5 vol.% Nonidet® P40-buffer solution, respectively.

With the used automated image processing system we measured 250 volumes recorded per printing buffer by the stroboscopic camera for droplets during flight (Fig. 4). Based on this we found the droplet diameter at optimal dispensing conditions to be dependent on viscosity and surface tension as well. For oligonucleotides as printing media the reproducibility of droplet diameter within one single channel was in general better than 1% independent from used printing buffer (Fig. 5). The droplet diameter and their CVs (per single nozzle) using protein media were measured by the mounted camera system on the TopSpot printer device. They were slightly higher (Fig. 6), but can be explained by tiny inhomogeneities of the hydrophobic surface coating of used microarray substrate and by differing solubility of proteins in buffer solutions. But with optimal dispensing conditions for both media CVs below 1% per single nozzle were achieved. Between all 24 nozzle of the printhead a CV better than 1.5% was measured, while using optimal dispensing conditions. The assumed measurement error of the stroboscopic camera method and the measured diameters of dispensed droplet ranging from 130 to 140 µm, resulting in a CV of measurement of about 1%. So the measured CVs of droplet diameter were close to the measurement error of the stroboscopic camera method.

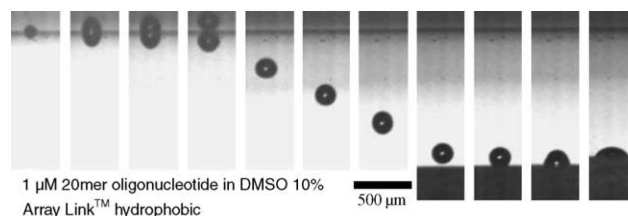

Fig. 4. Sequence of the dispensing process (left to the right) recorded by a stroboscopic camera: droplet formation and ejection out of a nozzle, droplet flight and droplet impact on the microarray slide.

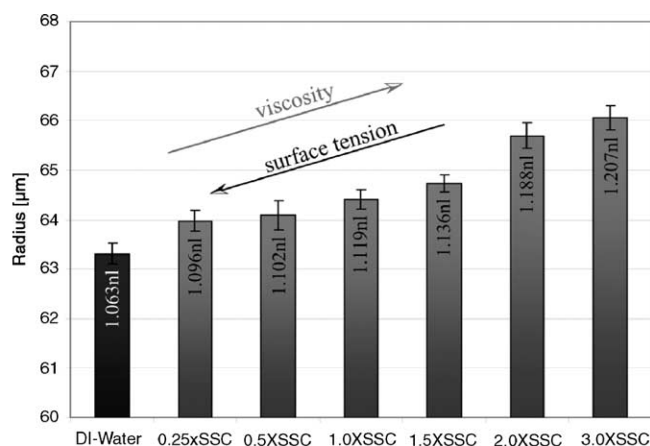

Fig. 5. Measured droplet radius (means out of 250 droplet ejections, error bars representing the standard deviation) and calculated droplet volumes of SSC buffers depending on viscosity and surface tension: The arrows indicate the increasing viscosity and surface tension of different SSC buffer concentrations.

One main reason for the small difference of 0.5% between different single nozzle data and 24 nozzles data is the hydrophobic coating of the nozzle surface. We suppose that tiny differences in the homogeneity of the hydrophobic layer have significant influence on the droplet release process. During the droplet ejection process less coated areas, first of all around the nozzle, get coated with printing solution, leading to a bigger tear-off edge diameter and in consequence to larger droplet volumes. Of minor importance should be variations in the manufacturing of the nozzles, due to the very precise used etching process.

The correlation between printing media properties and measured droplet diameter showed that the volume of dispensed droplets is highly depending on properties of printing media, mainly viscosity and surface tension. To achieve satellite-free droplet dispensing of all printing media the duration and amplitude of pressure pulse was adjusted to the specific printing media. In all dilution series of printing media the volume increased with increasing viscosity, but also with lower surface tension (Fig. 7). The higher viscosity results in higher friction between the fluid layers so that more fluid get pushed out of the fluid meniscus. A small filament

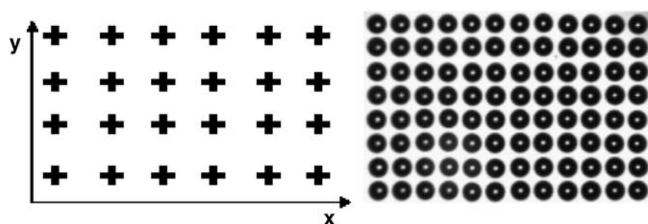

Fig. 6. Printing accuracy: On the left, overlaying of the measured *xy*-coordinates of spot centers from 16 sequentially printed arrays using the 24 nozzle printhead. Each cross indicates the center of a spot and the cross width the corresponding spot diameter. The deflection of all spots is less than 10 µm from regular 500 µm grid. On the right, a picture of a 250 µm spot spacing by interlacing printing is shown.

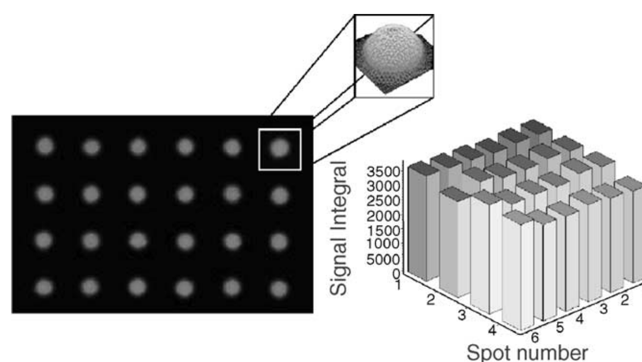

Fig. 7. Cy3-labeled 20mer (1 µM) oligonucleotides in 30% (v/v) DMSO on a microarray slide. The left side shows the fluorescence analysis of printed spots with a CV of 2.1% over all spots. On the top the distribution of fluorescence signal within one spot is shown in detail, demonstrating the high homogeneity of the printed spots.

of liquid is formed right before the droplet tears off caused by the surface tension of the printing fluid. Lower surface tension delays the formation of droplet, leading to higher droplet volume. Only the Nonidet® + 3× SSC buffer was an exception. In contrast to the other tested buffers the droplet volume first increased and then decreased with increasing surfactant concentrations.

The reason for this exceptional decreasing droplet volumes at very high surfactant concentrations, resulting in very low surface tension, remained unclear. In the experiments it was not possible to vary viscosity and surface tension independently. One possible way to assure which parameter is predominant would be a computer simulation of the printing process.

The pictures of printed arrays (right after each print), made by the mounted camera system of the TopSpot printer device, verified the results of droplets during flight (stroboscopic data). Both, spots of microarrays with oligonucleotides and also those with proteins, showed CVs of droplet diameters below 1.5% per printed array using optimal dispensing conditions. It also showed the high reproducibility of the whole printing process during printing hundreds of arrays. Comparing of all printed spots per array with all spots of the next printed arrays showed CVs below 1.6%, including the error of manufacturing dependent inhomogeneities of the hydrophobic coating of used microarray slides, which are leading to variations in spot diameter on the slide.

Also the highly correct position accuracy could be shown. The spot positions of a sequence of 16 printed arrays were measured geometric outline analysis using the automated image processing software NeuroCheck®. The deflection of spots was random and less than 10 µm from regular 500 µm grid. Higher spot densities on the substrates were generated successfully by printing between the spots of a printed array, so that with a 500 µm nozzle spacing a 250 µm spacing of spots was achieved (Table 1).The variation of piezo stack amplitude and the variation of initial actuation chamber

Table 1
Table of used printing buffer solutions for oligonucleotides printing and the corresponding droplet diameters with CVs, using the optimal pressure pulse for each printing medium

Buffer	Concentration	Diameter of dispensed droplets (μm)	CV (%)	Buffer	Concentration	Diameter of dispensed droplets (μm)	CV (%)
PBS (M)	0.0375	128.88	0.32	Glycerol (vol.%)	2.5	130.08	0.08
	0.075	128.88	0.22		5	131.98	0.33
	0.15	131.84	0.33		10	139.42	0.32
	0.225	134.18	0.29		15	141.32	0.55
	0.3	135.74	0.13		20	143.3	0.33
	0.45	135.32	0.17		30	142.58	0.30
SSC (M)	0.075	127.9	0.20	3× SSC × (M) betaine	0.2	129.22	0.22
	0.15	128.14	0.29		0.3	127.64	0.26
	0.3	128.8	0.19		0.4	127.8	0.22
	0.45	129.44	0.16		0.5	128.84	0.14
	0.6	131.40	0.24		1.0	134.28	0.33
	0.9	132.10	0.24		1.5	139.68	0.22
DMSO (vol.%)	5	129.14	0.19	3× SSC × (%) Nonidet®	0.0005	131.58	0.22
	10	128.22	0.10		0.001	136.02	0.10
	20	132.52	0.24		0.01	137.32	0.48
	30	136.74	0.29		0.05	135.54	0.32
	40	141.64	0.27		0.1	133.40	0.39
	50	143.58	0.25		0.5	122.80	0.81

Droplet diameters and CVs were measured during flight out of 250 droplet ejections using a stroboscopic camera.

volume enables generation of an ideal pressure pulse for stable and satellite-free printing of all used media. But based on one liquid the droplet volume is not modifiable through pressure modification, if one single droplet should be ejected, as it is possible in the direct displacement system described in [7]. By using different pressure pulses it was possible to print stable and reproducible media with up to 1 vol.% of surfactant solution (NP40 substitute). This represents a surface tension of approximately 26 mN/m. Lower surface tension led to leakage of fluid out of the nozzles, wetting of nozzle and with it less reproducible or completely missing droplet dispensing. Experiments with glycerol–water media showed that up to 30 vol.% glycerol was printable, which represents a viscosity of about 1.9 mPa. Higher concentrations led to no droplet ejection, because of the high fluidic resistance of these media.

Table 2
Table of used printing buffer solutions for BSA printing and the corresponding droplet diameters on a microarray substrate with CVs, using the optimal pressure pulse for each printing medium

Buffer	Concentration (M)	Diameter of droplets on the substrate	CV (%)	Buffer	Concentration (M)	Diameter of droplets on the substrate	CV (%)
PBS	0.05	239.74	0.53	ArrayLink	0.05	220.44	0.37
	0.1	241.00	0.42		0.1	210.66	0.51
	0.2	242.90	0.66		0.2	210.78	0.63
	0.3	244.76	0.42		0.3	213.76	0.66
	0.4	244.86	0.81		0.4	219.54	0.67
	0.5	245.80	0.34		0.5	219.90	0.48
SSC	0.05	252.22	0.52	Borate	0.05	208.44	0.67
	0.1	255.96	0.62		0.1	209.32	0.47
	0.2	254.66	0.68		0.2	209.64	0.53
	0.3	257.70	0.49		0.3	212.10	0.64
	0.4	256.16	0.47		0.4	213.88	0.81
	0.5	261.70	0.84		0.5	218.32	0.81
Sodium-carbonate	0.05	225.84	0.28				
	0.1	261.70	0.30				
	0.2	218.62	0.31				
	0.3	218.90	0.59				
	0.4	219.40	0.17				
	0.5	220.72	0.43				

Droplet diameters and CVs out of 25 pictures of printed arrays on the microarray slide using the mounted camera system of the TopSpot printer device.

Table 3
Overview table of the results of the reproducibility experiments showing the high reproducibility of the system with camera data as well as with a typical microarray method

CV of single nozzle by stroboscopic camera (%)	1.0
CV over the printhead by stroboscopic camera (%)	1.5
CV array to array by camera method (%)	1.6
CV of print-head using fluorescence method (%)	2.1

The outstanding performance of this printhead was also verified in microarray experiments using the fluorescence based quantification of the dispensed volumes with Cy3-labeled oligonucleotides and Cy5-labeled antibodies in different printing buffers. We found the CV of fluorescence signals over all 24 spots to be better than 2.1% depending on used printing buffer (Table 2). The different results of CVs compared to the camera data can be explained by environmental influence factors, e.g. humidity or temperature, and measurement errors due to the fluorescence method on microarray slides, e.g. quenching of used fluorochromes or salt out effects in spots. With it the camera results were verified in a typical microarray experiments and showed that the system is suited for high quality microarray production (Table 3).

4. Conclusion

We have investigated the correlation between satellite free droplet release and liquid viscosity in a highly parallel, pressure driven nanoliter dispenser. In extensive studies we found that for liquids of different viscosities the duration of the pressure pulse is the predominant effect compared to pressure amplitude. By variation of pressure pulse amplitude and duration we were able to dispense stable and reproducible up to 1.9 mPa of viscosity, which is equivalent to the viscosity of 30 vol.% glycerol/water. The results suggest that higher viscosities could be dispensed by longer duration of pressure pulse, but this was not possible with the used experimental set-up. Surface tensions below 26 mN/m led to leakage of fluid out of the nozzles, wetting of nozzle, even before actuation. To dispense media with lower surface tension, the hydrophobic coating of the printhead has to be improved.

Experiments with oligonucleotides as well as with different proteins showed ascertained carry-over and cross-talk free printing of DNA and protein microarrays. With it for our system we solved a prime critical point of usage in microarray production, enabling high quality whilst high throughput microarray fabrication.

For oligonucleotide printing we measured CVs of droplet diameter to be better than 1% within one single dispensing nozzle. Using protein solutions as printing media led to slightly higher CVs, but as for the oligonucleotides below 1% per single nozzle. Within all 24 nozzles of a printhead we found CVs to be better than 1.5% for each used printing buffer using optimal dispensing conditions. The tiny differences between single nozzle and all nozzles of a printhead are supposed to be influenced by in inhomogeneities of the hydrophobic coating around the nozzle surface. So ultra-hydrophobic coating with protein repelling properties would be favorable.

5. Outlook

The future work will be the evaluation of this results with other biological molecules of higher complexity and molecular weight. Therefore an adaptation of the electronic driver of the piezo actuator for increased pressure pulse duration is necessary. To assure which parameter is predominant a computer simulation of the printing process will be established in the near future. A pressure sensor in the piston will be integrated into the system to get on-line pressure data during droplet dispensing process. The hydrophobic coating of the nozzle area has to be improved to achieve a stable and reproducible droplet ejection even with higher complex printing medias. The coupling efficiency of printing media by using different printing buffers should be evaluated to find an optimal printing buffer system for microarray production with the TopSpot system, that means microfluidically as well as biochemically. Printheads with different nozzle diameters will be fabricated to achieve lower droplet volumes, e.g. for printing on very hydrophilic substrates without mixing of spots.

Acknowledgements

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Biographies

Oliver Gutmann received his DiplIng degree in biotechnology from the Ecole Supérieure de Biotechnologie (ESBS) in Strassbourg (France) in 2001. He made his diploma thesis at Genescan Europe AG (Germany) in 2001 entitled "production of protein microarrays using TopSpot". Afterwards he worked as a R&D engineer at Genescan Europe AG. Since 2002, he is working towards a PhD degree at the University of Freiburg, Institute of Microsystem Technology (IMTEK), Chair for MEMS Applications. Oliver's research interests include microarrays, microfluidics and microsystem technology.

Remigius Niekrawietz studied microsystem technology at the University of Freiburg, Germany. In 2003 he finished his diploma thesis and received his DiplIng degree in microsystem technology. Since March 2003 he is working as a R&D engineer on a PhD project in the laboratory for MEMS applications at the Institute for Microsystem Technology (IMTEK). His thesis and current work is in the field of life sciences, where he develops a control loop for the automatic adaptation of the optimal parameter for unknown printing media properties for the production of microarrays.

Ruben Kuehlewein was born in Freiburg, Germany in 1977. He received his graduate in civil engineering in microsystem technology in 2003 at the Institute of Microsystem Technology (IMTEK) at the University of Freiburg, Germany. His diploma work was in the field of life science, where he investigated and improved the (mass) fabrication of protein micro-arrays according to the principle of TopSpot 4. In October 2003 he founded the limited company Invention Agency Ltd., whose primary objective is the development and distribution of products in the field of engineering (especially of microsystem technology).

Chris P. Steinert was born in Germany in 1975. He received his DiplIng degree in microsystem technology in 2001 at the University of Freiburg, Germany. Since January 2002 he is working as a R&D engineer on a PhD project in the laboratory for MEMS applications at the Institute for Microsystem Technology (IMTEK). His diploma and current work is in the field of life sciences, where he develops a dispensing device for the (mass) production of microarrays.

Bas de Heij obtained his MSc degree in the year 1996, from the university of Twente (NL) on work entitled 'micromachining in stainless steel'. For his PhD work he joined the group at the IMT in Neuchâtel (CH) from Prof. De Rooij. His PhD thesis was finished in the year 2000 and is entitled 'development of a micromachined vaporizer for inhalation drug therapy'. In that same year he joined the group of Prof. Zengerle. His main interest is the development of methods and equipment for the highly parallel generation of nL and sub-nL droplets.

Roland Zengerle holds the chair in MEMS applications at the Institute of Microsystem Technology (IMTEK) at the University of Freiburg, Germany, and he works in close cooperation with the Institute for Micro and Information Technology of the Hahn-Schickard-Society. His research is focused on microfluidics and covers topics like miniaturized and autonomous dosage systems, nanoliter and picoliter dispensing techniques, lab-on-a-chip systems, micro reaction technology as well as micro- and nanofluidics simulation. Dr. Zengerle co-authored more than 120 technical publications and 20 patents. He serves on the international steering committee of the IEEE-MEMS conference as well as on the technical program committee of the Bi-Annual Actuator Conference. Dr. Zengerle is the European editor of the newly launched Springer Journal of Microfluidics and Nanofluidics.

Martina Daub finished her diploma thesis in 1996 in biology (immunology and microbiology). She earned a PhD (1996–1999) at the 'Max-Planck-Institute for Molecular Physiology' in Dortmund (signal transduction, basic cancer research). A biennial postdoc was performed at the Bayer-AG in the department of Research Toxicology in Wuppertal (molecular toxicogenomics). Since August 2001 she has been working in the position of "Head of Biomolecular Applications" at the IMTEK (Institute for Microsystem Technology) at the University of Freiburg in the field of microfluidics. Her current fields of interest are: microfluidics, microsystem technology, microarrays, lab-on-a-chip, molecular biology, pharmacy, biotechnology, molecular and cellular biology, biochemistry, immunology and toxicology.