

## **Microfluidics and Beyond - Devices for Applications in Biotechnology -**

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### **ABSTRACT**

For the performance of certain analytical and diagnostic tasks in modern Life Science applications high throughput screening (HTS) methods are essential. Miniaturization, parallelization and automation allow to decrease consumption of expensive materials and lead to faster analyzing times. The miniaturization of total assay volumes by the use of microtiter plates as well as the microarray technology have revolutionized the field of biotechnology and Life Sciences. Neither printing of microarrays with droplet volumes of several picoliters, nor handling of precious enzymes in the upper nanoliter range can be accomplished with traditional liquid handling devices like air displacement pipettes. The development of novel low volume liquid handling devices, which are subject to current research, addresses the diverse requirements shifting steadily to lower volumes. Various novel non-contact dispensing methods in the nanoliter and picoliter range are presented and classified according to their working principles like air displacement and direct displacement methods (TopSpot<sup>®</sup>, NanoJet<sup>™</sup>, Dispensing Well Plate<sup>™</sup>). Properties of the various methods are compared in terms of flexibility, integration density, speed of operation, precision, addressable volume range and amenability to multi-parallel operation.

By integrating processing steps of biological assays within these novel non-contact dispensing devices multifunctional Lab-on-a-chip (LOAC) devices can be developed. A prototype of such a flexible and modular application platform was developed. This platform enables to perform various processing steps (e.g. PCR, post-processing) in one chip with subsequent probe transfer into another chip with a different functionality (e.g. detection). This basically points into the direction to reach new functionalities by combining advantages of novel low volume liquid handling devices with LOAC functionality.

### **INTRODUCTION**

Microsystem Technology is an emerging key technology of the current century due to the permanently increasing demand for smaller, more economical and higher integrated systems with microfluidics as one of the major application areas. As an engineering science microfluidics deals with the handling of fluids (liquids or gases) in “micro” amounts (microliter, nanoliter, picoliter range) or with “micro” sized devices or with low power consumption. Due to increased surface-to-volume ratios in microfluidics it is possible to utilize physical phenomena that are not available or relevant to that extent in a macro environment (e.g. capillary forces for transportation of liquids, rapid heat exchange, short diffusion distances, adhesion and similar effects). At the same time effects like gravity, inertia and turbulences are negligible in that volume ranges. The field of Life Sciences offers challenging applications because of the continuing trend towards miniaturization, parallelization and automation of biological and chemical processing and

detection protocols. The technological advantages are evident: reduced amounts of reagents and with it costs; small size and weight of devices; short time-to-results with drastically reduced analyzing times; enhanced system integration and automation enabling higher throughput. The *FlowMap* survey identified drug discovery, medical diagnostics and therapeutic devices to be the most promising fields in the Life Science area with an expected overall annual growth rate for microfluidic technologies of more than 30% per annum [1].

Reduction of total reaction volumes and therefore scaling down the volumes of liquid handling processes from the milli- and microliter range to the nano- and picoliter range is a condition precedent and can only be realized through precise control of small amounts of liquid. Traditional liquid handling devices like air displacement pipettes neither fulfill the need for accurately printing droplet volumes of several hundred picoliters to fabricate microarrays nor for handling of enzymes or basic compounds for drug design in the upper nanoliter range. Capillary and adhesive forces at the tip of those pipettes when inserting the tip into the medium result in large errors when moving to submicroliter range and also provide the risk of cross-contamination. For industrial applications these errors are intolerable [2].

The development of novel low volume liquid handling devices, which are subject of current research remove these restrictions by new dosing principles that dispense liquid in a contact-free manner. With different dosage technologies developed for diverse applications various needs can be met. The general principle of all presented technologies for nanoliter and picoliter dosage is the delivery of reagents as free-flying droplets or as jets of liquid. The central features of all technologies described are that no carry-over and cross-contamination occur and that all kinds of substrates are suitable without damage of the surface due to the contact-free dosage. Most of the devices also exhibit media independent performance.

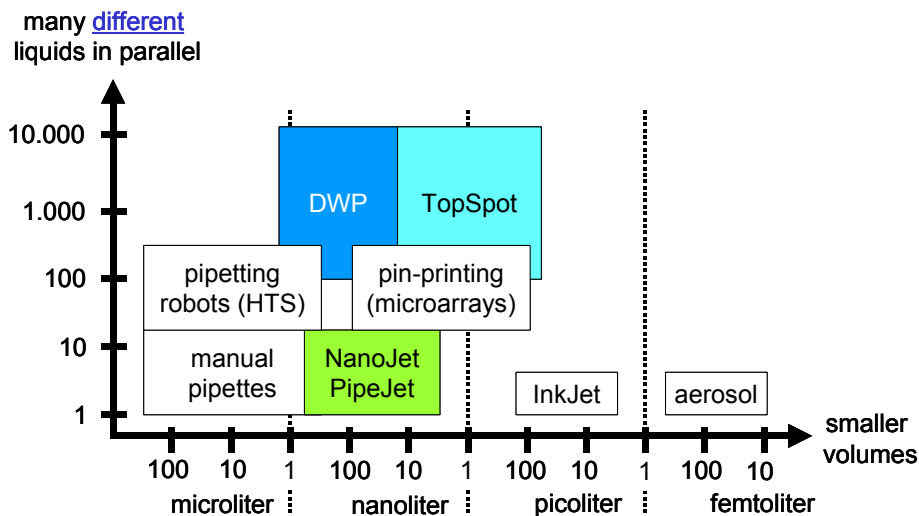
## EXPERIMENTAL AND RESULTS

Different challenges for low volume liquid handling in the heterogeneous field of applications demand for different solutions regarding addressable volume range, flexibility, integration density, speed of operation, precision and amenability to multi-parallel operation. Thus various novel non-contact dispensing methods in the nanoliter and picoliter range are developed. In the following paragraphs the technologies are described and classified according to their working principles and to their fields of applications in biotechnology.

TopSpot<sup>®</sup>, NanoJet<sup>™</sup>, PipeJet<sup>™</sup> and DWP<sup>™</sup> are different with respect to the addressed volume range and the number of different liquids that can be dispensed simultaneously. The technologies are partly able to dispense several thousands of substances within less than a second. The volume range of the devices as well as the degree of parallelism is shown in Fig. 1 and discussed in the following.

### **TopSpot<sup>®</sup> Technology**

The challenge to develop the TopSpot<sup>®</sup> technology came up due to the broad use of microarrays as analytical tools in biotechnology and in near future in molecular diagnostics [3]. Microarrays allow to analyze the content of complex fluids for thousands of different parameters



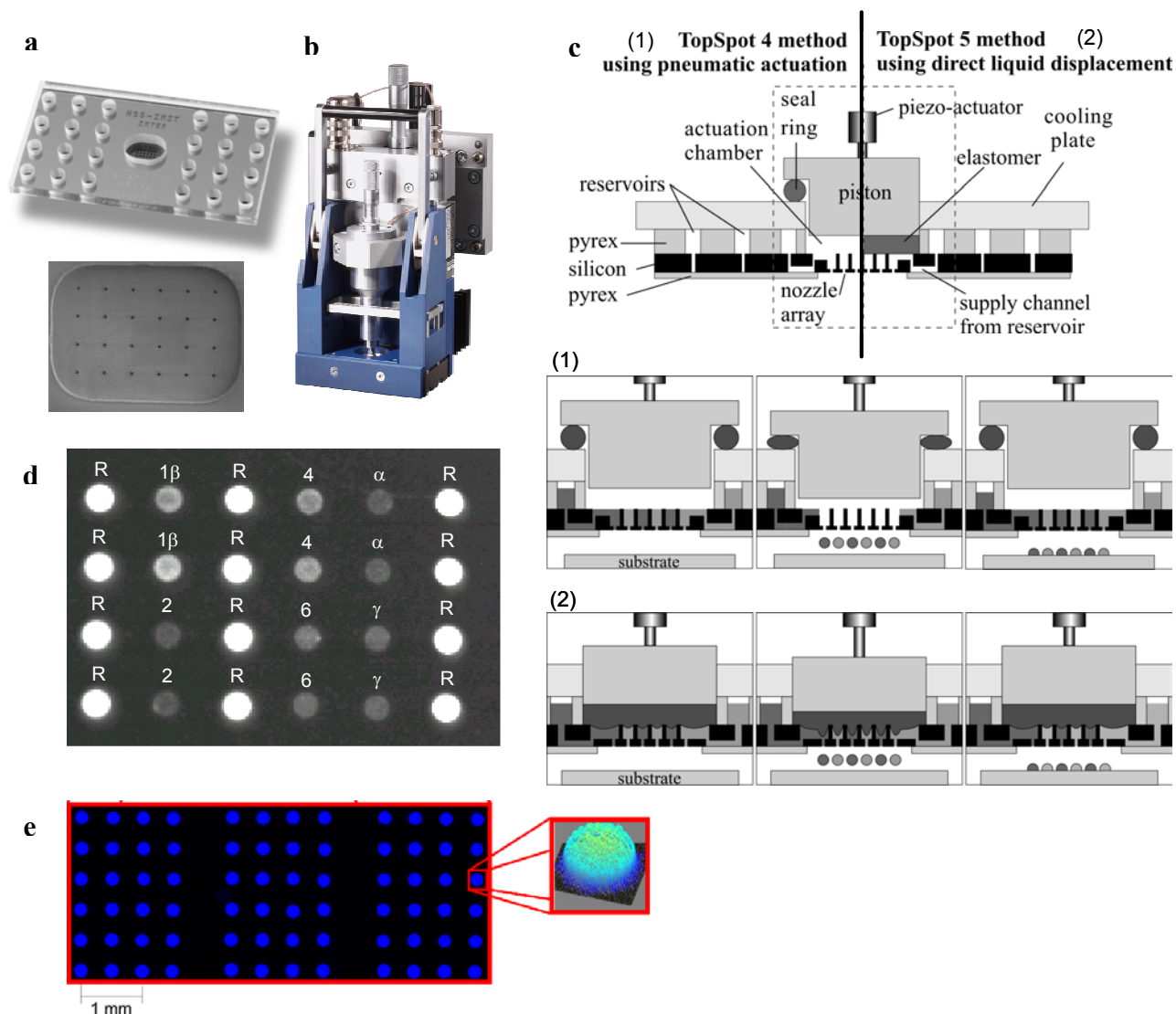
**Figure 1: Map of nanoliter and picoliter dispensing.** The focus of research is higher parallelization of smaller volumes. Technologies like TopSpot<sup>®</sup>, NanoJet<sup>™</sup>/PipeJet<sup>™</sup> and DWP<sup>™</sup> fill the gap between existing liquid handling devices regarding volume range and degree of parallelization.

in one experiment. This is based on specific binding of those molecules to probes that are fixed on a substrate as spots in a predetermined matrix. TopSpot<sup>®</sup> technology is dedicated to the economical production of such microarrays. It is suited for the massive parallel dosage of different biological and chemical reagents in a very small array pitch of 500  $\mu\text{m}$  to apply them onto a substrate.

With TopSpot<sup>®</sup> technology a reliable and robust method is given for highly parallel printing suitable for high throughput fabrication of microarrays with good spot quality. The use of this method in comparison to a traditional pin printer 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 carry-over and the high reproducibility of the printing process.

The core of the TopSpot<sup>®</sup> operation principle is a microfabricated printhead (silicon/glass technology) (Fig. 2a). This printhead facilitates the dosing of many different liquids at a very tight pitch at the same time. The number of dispensed droplets printed at the same time depends on the used printhead type. Printheads with 24, 96, and 384 reservoirs respectively have been successfully realized and operated. The first step in operating the TopSpot<sup>®</sup> arrayer is to fill different reagents into reservoirs which are arranged in an ordinary microtiter plate format (e.g. 2.25 mm) for standardization purposes. The liquid in the reservoirs (maximum filling several  $\mu\text{L}$ ) is usually sufficient for 1,000 to several thousand prints and is transported by capillary forces to the central nozzle part. The nozzles are usually arranged in a 500  $\mu\text{m}$  pitch according to the aimed matrix for low and medium density microarrays. The integrated format conversion in the printhead is one of the key factors for increasing the speed and for the ease of handling of the device. Therefore far less complicated automation concepts can be used for production equipment if TopSpot<sup>®</sup> technology is applied.

For operation the printhead is placed in a print module (Fig. 2b) which contains a piezo actuation system. Through an adjustable stroke of this actuator a pulse of high pressure is



**Figure 2: TopSpot® Technology.** (a) Photograph of printhead with 24 reservoirs (top) and close-up view of the nozzles (bottom). (b) Print module to actuate one printhead. (c) Working principle of TopSpot® with pneumatic actuation (1) and direct displacement actuation principle (2). Cross section of both actuation methods (top) and experimental set-up (bottom). The filled printhead is placed into a print module. Movement of the piston causes a compression of air (1) and displacement of elastomer (2), respectively. This pressure ultimately results in droplets to be ejected out of the nozzles. (d) Array of antibodies incubated with a mixture of labeled corresponding cytokines, R = directly Cy5-labeled BSA; 1 $\beta$  = anti-interleukine  $\beta$  (IL-1 $\beta$ ), 2 = anti-IL-2; 4 = anti-IL-4, 6 = anti-IL-6,  $\alpha$  = anti-tumor necrosis factor  $\alpha$ ,  $\gamma$  = anti-interferone  $\gamma$  (courtesy of Zeptosens). (e) Fluorescent image of 1  $\mu$ M Cy3-labeled 20mer oligonucleotide.

generated affecting all nozzles at the same time thereby causing each nozzle to eject a droplet. Currently there are two operating actuation methods:

- TopSpot 4 principle – pneumatic actuation (Fig. 2c (1))
- TopSpot 5 principle – direct displacement actuation (Fig. 2c (2))

In the TopSpot 4 principle an impulse on the piezo actuator moves a piston (Fig. 2c). This generates a pressure pulse in an air chamber above the nozzles. This highly dynamic pressure pulse affects all nozzles simultaneously, causing them to eject one droplet each. The volume of these droplets is typically 1 nL. Other volumes can be achieved by using different nozzle diameters.

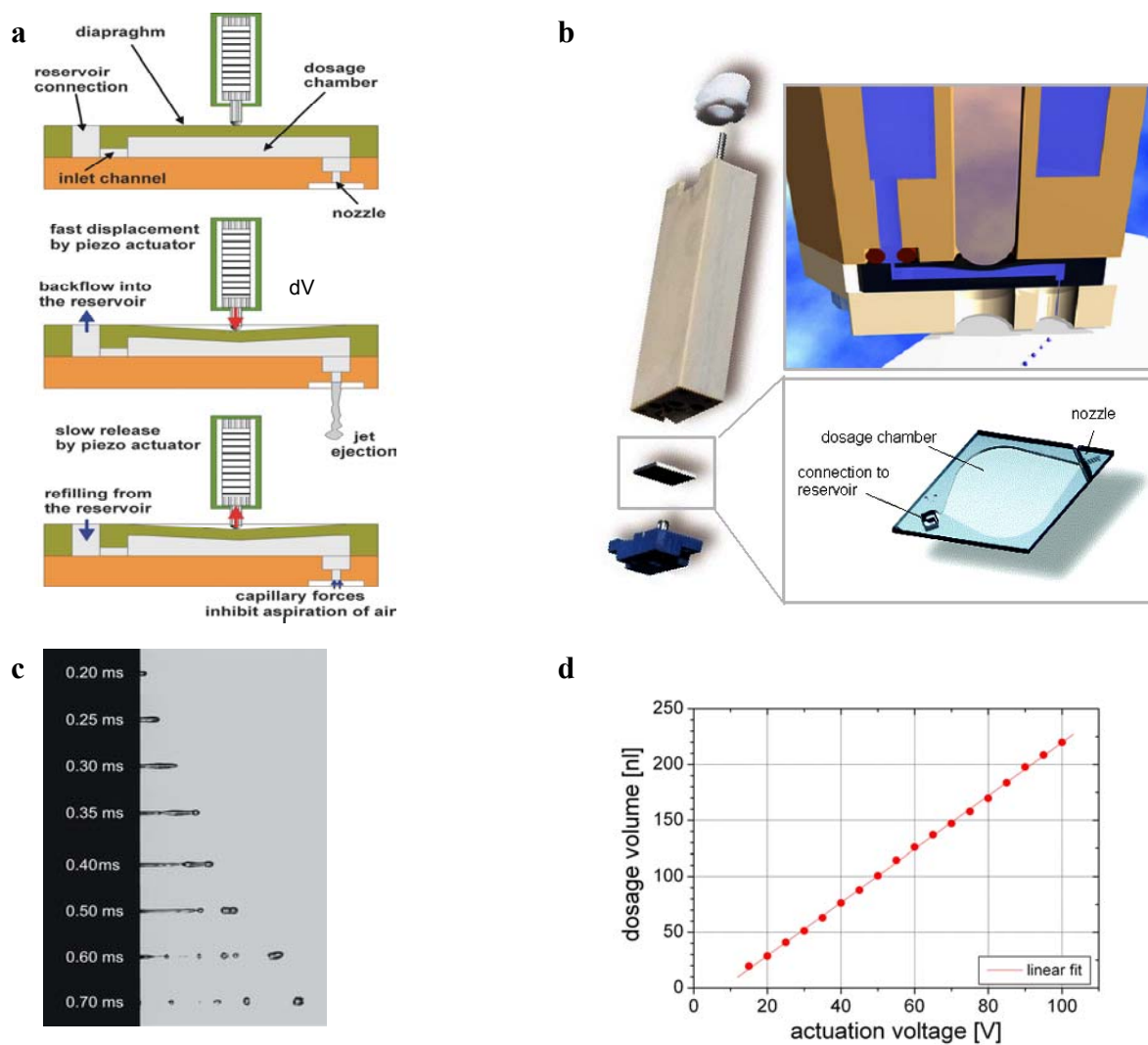
The TopSpot 5 principle [4] uses an incompressible elastomer instead of the air chamber (Fig. 2c). Movement of the piston causes the elastomer to be squeezed into the nozzles. The liquid in the nozzle chamber is displaced and therefore a droplet is ejected from each nozzle. Using this direct displacement method it is possible to set the ejected droplet volume and speed by the stroke and speed of the piezo actuator. The achievable volume range is between 60 pL and 1,400 pL (1.4 nL).

The print module (Fig. 2b) is the smallest unit to operate the TopSpot® technology. It can be placed in numerous automation environments. The modular concept applied for all systems allows easy upgrade from microarray design (research application) to high throughput fabrication (industrial mass production). Currently there are three different configurations available that differ in the number of printheads that can be used with one setup, in the existence of integrated quality control and in the number of mounted substrates.

The fabrication of microarrays does not end with the transfer of the interesting analytes onto the substrate but consists of multiple steps: the preparation of the substrates, the deposition of the probe molecules and the coupling of these molecules to the substrate including the subsequent washing to dispose unbound molecules. Besides the employed printing technology further factors influence the quality of microarrays: the surface properties of the substrate, the printing media (e.g. buffer solutions, supplements, probe molecules) and the printing environment (e.g. temperature, humidity). To gain highly reproducible microarrays of high quality all these parameters have to be optimized and adopted. To guide TopSpot® users 'standard operating procedures' (SOP's) are established for a multitude of probe types (DNA, proteins, cells) in combination with medium composition and a host of substrates. Moreover the optimal coupling method has to be evaluated. The optimal conditions for the subsequent hybridization of complementary molecules out of the complex liquid that should be analyzed have to be found. In ref. [5] a detailed list of print media for printing of oligonucleotides is compiled.

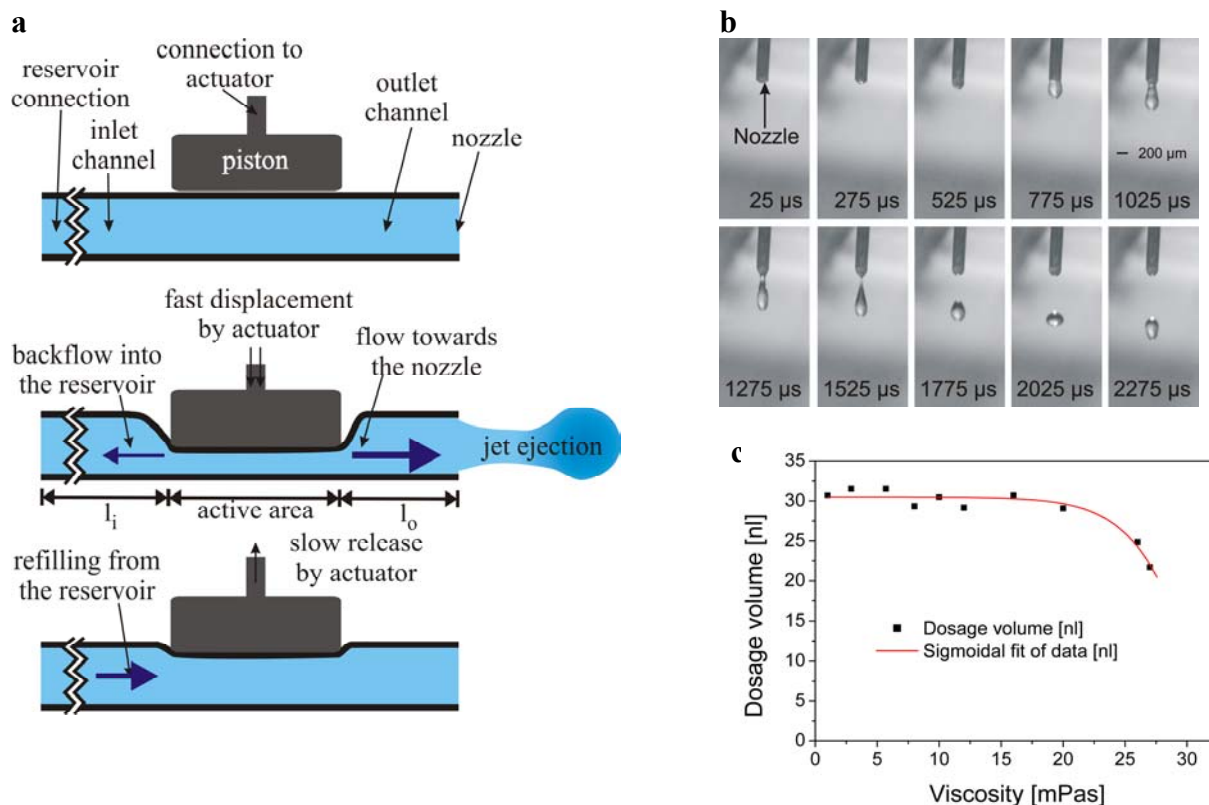
### **NanoJet™ and PipeJet™**

Nowadays adjustable liquid handling in the microliter range is common standard. Manual pipettes and pipetting robots are employed for this. Accurate pipetting of small volumes of liquid is the basic requirement for the performance of robust and reliable biotechnological assays. The invention of a technology for the accurate dosage of smaller volumes drives the Life Sciences to even higher miniaturization and with it parallelization of assays in e.g. drug development, diagnostics and therapeutics making use of advantages that accrue from involving microfluidical effects. The task that had to be performed for the dosage of nanoliters in an adjustable manner for volumes from 5 to 200 nL were to dose with high accuracy (CV below 5%) and to dose independently of the media properties (such as surface tension and viscosity). These demands can be met with the invention of the NanoJet™ technology that was developed in cooperation with the *Eppendorf AG*.



**Figure 3: NanoJet™ Technology.** (a) Schematic of the dispensing cycle. The liquid is ejected as a free-flying jet out of the nozzle by direct displacement of the silicon membrane caused by a piezo stroke. The refilling after actuation is driven by capillary forces. (b) Micromachined NanoJet™ dosage chip (bottom right) mounted into a cartridge holding the printing medium (left). Schematical cross section of the cartridge holding the dosage chip. (c) Time-resolved stroboscopic sequence of the jet ejection. (d) NanoJet™ dosage volume is adjustable by piezo stroke. Dosage volume ranges between several nL to several hundreds of nL.

The Nanojet™ principle is based on a fast mechanical displacement of liquid from a microstructured dosing chamber (Fig. 3a, 3b). The displaced volume can be adjusted continuously by the displacement of a piezostack actuator. A defined part of the liquid is expelled as a free flying jet – a so-called nanojet – from a micronozzle. At the same time the remaining part of the displaced volume flows from the dosage chamber back into the reservoir. Due to the well-defined flow resistances that are present at the inlet channel and at the nozzle, reproducible dosage, irrespective of media properties, is achieved. The Nanojet™ principle is the first one which enables the user to dispense media with highly different characteristics (viscosity range of 1 - 100 mPas) at a very high level of accuracy (data not shown). Upon slow release of the actuator the refilling of the dosage chamber is accomplished by capillary forces at the nozzle. Apart



**Figure 4: PipeJet™ Technology.** (a) Schematic of the dispensing cycle. But instead of a micromachined dosage chip (NanoJet™) a clamped elastomer tube (inner diameter 200  $\mu$ m, wall thickness 25  $\mu$ m) is applied. (b) Time-resolved stroboscopic images of an ejection process. (c) Dosage volume versus medium viscosity at a frequency of 0.1 Hz with 50  $\mu$ m stroke and different glycerol/water dilutions.

from manually operated pipettes and dispensers, NanoJet™ dispenser systems were combined into multi-channel systems. These are especially well-suited for use in automated pipetting and analyses systems. In the framework of an industrial cooperation a prototype with 16 independently controllable dispensers which can be used to fill microtiter well plates was developed [6,7].

Further development of the NanoJet™ technology led to the PipeJet™ technology by performing consequent simplification and optimization. Instead of a microstructured dosage chip and with its complex micro technical etching processes, a disposable elastic polymer tube with a defined inner diameter is applied. Therefore the PipeJet™ dispenser is much more cost effective. Actuation for non-contact dispensing in the nanoliter range is performed by the highly dynamically stroke of a piezo actuator. A piston squeezes the tube and leads to direct displacement of liquid out of the tube. The actuation principle is shown in Fig. 4a and is, like NanoJet™, in a wide range independent on liquid properties (e.g. viscosity range: 1-20 mPas). The working principle has been proven by stroboscopic and gravimetric measurements (Fig 4b, 4c) [8].

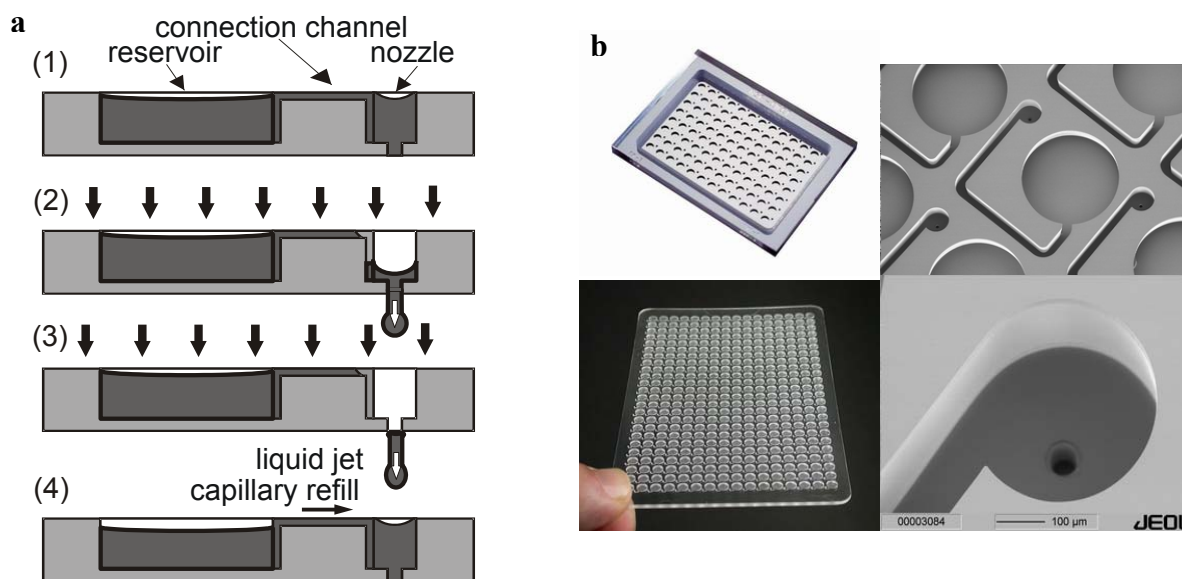


## Dispensing Well Plate™

Microtiter plates are standard tools in high-throughput screening in pharmaceutical industry (e.g. drug discovery). Enormous amounts of different reagents have to be combined by time-consuming pipetting steps. The smallest volume that can be accurately dosed is the basic unit for the total volume of such combinatorial chemistry processes. Going smaller with these volumes could drastically reduce total assay volumes and with it costs in many fields of research and development. Besides liquid handling for combination of various liquids storage of these liquids is another big issue.

The Dispensing Well Plate (DWP™) system addresses both issues. It was developed especially for dispensing of reagents into microtiter plates. The focus of this technology lies in the simultaneous and thus highly parallel dispensing of up to 1536 channels of a predefined fixed volume in the nanoliter range (e.g. 50 nL).

For applying the DWP™ method the various reagents to be dispensed are filled into individual reservoirs that are arranged in a microtiter plate format (Fig. 5b). Driven by capillary forces each liquid is transported through a micro channel to the corresponding micro nozzle containing a defined volume. The crucial parameter for dosage volume is the geometry of the nozzle chamber. By applying a pneumatic pressure pulse to the whole plate the total liquid in the nozzle chamber is dispensed. The dosage volume is in a wide range independent of the duration and the amplitude of the applied pressure pulse. The robustness of this method is further supported by widely dosage volume independency of liquid properties like viscosity, density and surface tension. However it



**Figure 5: DWP™ Technology.** (a) Dosage principle shown for one single dispensing unit of a DWP™ at successional stages (1) before dispensing (2) during jet ejection of a fixed volume given by nozzle chamber geometry (3) after ejection (4) after capillary refilling of nozzle chamber. (b) Photograph of a DWP™ prototype with 96 dispensing units micromachined in silicon (top, left). SEM photographs of one silicon dosage unit (reservoir, nozzle, channel) (top, right) and close-up view of the nozzle (bottom, right). Photograph of a full-size DWP™ prototype with 384 dispensing units manufactured in PMMA.



has to be noted that the dispensed volume is constant and cannot be adjusted during operation. This is due to the fact that the nozzle volume is defined during micro fabrication of the device.

The extremely low coefficient of variation of only 3 % at a dosing volume of an aliquot of 50 nL gives this system a very high potential for increasing the quality and throughput in high-throughput screening. In principle the DWP<sup>TM</sup> method enables an arbitrary number of dispensing units to be operated in parallel. The dosage volumes can be adjusted during fabrication to be between 10 nL and several 1,000 nL. Depending on the volume of the reservoirs the dosage process can be repeated thousand times with one single filling. DWP<sup>TM</sup> dosage devices with up to 384 parallel channels and a dosage volume of 50 nL have been already tested successfully [9,10].

## DISCUSSION AND CONCLUSION

The presented novel low volume liquid handling devices were developed to cover the needs of modern chemical and biochemical methods and protocols in terms of further miniaturization and parallelization. To fulfill the diverse requirements these novel devices address the steady shift towards lower volumes. The technologies have a lot in common: (1) So all presented technologies act in a non-contact manner. (2) The dosage volume of all presented methods is provided in the nanoliter and picoliter range. (3) Microfluidic effects are employed to simplify actuation principles. (4) In respect to standardization purposes formats were chosen that match with both interfaces to potentiate filling of the device with a standard pipetting robot as well as transfer of droplets or jets in a spacing corresponding to microtiter plate formats. (5) Emphasis was placed on simplicity and robustness of all dispensing methods.

Beyond the common properties each technology meets its specific range of demands regarding fixed or adjustable volume, precision, parallelism or at least amenability to multi-parallel operation, integration density, flexibility, dosage frequency, applicable media, actuation principles, etc.. Which technology has to be chosen for a special given application depends on the kind and weighting of specifications predetermined by application. Each technology was invented and developed application-oriented for concrete user needs: Thus TopSpot<sup>®</sup> technology was developed for highly parallel printing of microarrays with droplet volumes of several 100 pL to 1 nL, each droplet consisting of another analyte. The characteristic of NanoJet<sup>TM</sup> to precisely dispense an adjustable volume in the upper nanoliter range was especially developed to dispense expensive reagents like enzymes irrespective of medium properties. The key application for DWP<sup>TM</sup> is high-throughput screening to replicate the content of one microtiter plate into a multitude of receiving plates combined with provision of a solution for storage of substance libraries to increase application flexibility. Extensive experiments have been performed to verify the high precision and accuracy and satellite-free droplet and jet release. Tests with biologically relevant materials showed the applicability of these devices in the field of Life Science. Table I gives an overview of the technological specifications of the presented technologies. For further applications adaptations and advanced developments of the shown methods are feasible.

The development of liquid handling devices still continues. In near future we will carry on working on simplification methods and improvements regarding employed materials to reduce costs and to fabricate disposable devices. Furthermore higher integration levels have to be reached. On the one hand this could be reached by advanced automation, on the other hand, where applicable, by implementation of higher parallelization.

**Table I:** Overview of technical specifications of presented contact-free liquid handling technologies. TS 4 – TopSpot<sup>®</sup> with pneumatic actuation; TS 5 – TopSpot<sup>®</sup> with direct displacement actuation. The devices are used for various biotechnological applications depending on the different demands in terms of flexibility, integration density, speed of operation, precision, addressable volume range and amenability to multi-parallel operation.

	TopSpot <sup>®</sup>		NanoJet <sup>™</sup>	PipeJet <sup>™</sup>	DWP <sup>™</sup>
	TS 4	TS 5			
<b>dosage volume variation</b>	fixed volume	adjustable	adjustable, viscosity indep.	adjustable, viscosity indep.	fixed by geometry of nozzle chamber
<b>dosage volume / range</b>	1 nL	100 pL to 1.4 nL	5 to 200 nL	5 to 100 nL	10 to 1,000 nL (one fixed vol.)
<b>dosage frequency</b>	2 Hz (for 1 nL)	10 Hz (for 200 pL)	10 – 50 Hz	up to 50 Hz	1 Hz (for 50 nL)
<b>volume CV (one nozzle)</b>	< 1 %	< 1 %	< 3%	< 2%	< 2% depends on microstructuring technology
<b>volume CV (all nozzles)</b>	< 2 %	< 2 %	< 5%	parallelism to be implemented	< 3%
<b>number of parallel channels</b>	24 / 96 / 384 (printhead design)	24 (to be extended)	if modular → 16	1 (in principle unlimited)	384 (in principle unlimited)
<b>reservoir pitch</b>	4.5 or 2.25 mm	4.5 mm	if modular → 9 x 18 mm individually addressable	use of external cartridges (2.25 mm possible)	4.5 or 2.25 mm
<b>nozzle pitch</b>	500 μm	500 μm	9 x 18 mm possible	2.25 mm possible	4.5 or 2.25 mm
<b>approved printing media</b>	aqueous solutions, glycerol, solvents, DNA, proteins, etc.	aqueous solutions, glycerol, solvents, DNA, proteins, etc..	aqueous solutions, solvents, lubricants, adhesives, particle laden liquids	aqueous solutions, oil, solvents, lubricants, adhesives, particle laden liquids	aqueous solutions, solvents, DMSO
<b>viscosity range</b>	1 - 3 mPas	1 - 22 mPas	0.7 - 100 mPas	1 - 20 mPas	1 - 10 mPas
<b>surface tension</b>	25 - 76 mN/m	25 – 76 mN/m	20 - 76 mN/m	20 - 76 mN/m	30 - 76 mN/m
<b>marketing status</b>	commercially available	beta-testing phase	commercially available	beta-testing phase	development phase

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## REFERENCES

1. J. Ducrée, R. Zengerle; „FlowMap – Microfluidics Roadmap for the Life Sciences”; edited by J. Ducrée and R. Zengerle, ISBN code: 3-8334-0744-1, 2004, Books on Demand, Norderstedt, Germany (also available at [www.microfluidics-roadmap.com](http://www.microfluidics-roadmap.com)).
2. P. Koltay, J. Ducrée, R. Zengerle; “Nanoliter and Picoliter Liquid Handling”; edited by Edwin Oosterbroek and Albert van den Berg; Elsevier; Elsevier Science, Amsterdam; ISBN code: 0-444-51100-8; September 2003; pp. 151-171.
3. B. de Heij, M. Daub, O. Gutmann, R. Niekrawietz, H. Sandmaier, R. Zengerle; „TopSpot – Highly Parallel Dispensing of Chemical and Biological Reagents”; Analytical and Bioanalytical Chemistry; Vol. 378, 2004; pp. 119-122.
4. C.P. Steinert, I. Goutier, B. de Heij, O. Gutmann, M. Daub, H. Sandmaier, S. Messner, R. Zengerle; “An improved 24 channel picoliter dispenser based on direct liquid displacement”; Proc. of IEEE-Transducers 2003, 8.-12.06.2003, Boston, USA, 2003, pp. 376-379.
5. O. Gutmann, R. Kuehlewein, S. Reinbold, R. Niekrawietz, C.P. Steinert, B. de Heij, R. Zengerle, M. Daub; “A highly parallel nanoliter dispenser for microarray fabrication”; *in press* Biomedical Microdevices 6:2. 2004, pp. 131-137.
6. R. Steger, P. Koltay, G. Birkle, T. Strobel, H. Sandmaier, R. Zengerle; „A Two-Dimensional Array of Piezostack Actuated Nanoliter Dispensers”; Actuator 2002; 10-12 June 2002; Bremen; Germany, pp. 537-541.
7. P. Koltay, R. Steger, G. Birkle, H.-C. Huang, H. Sandmaier, R. Zengerle; “Microdispenser array for highly parallel and accurate liquid handling”; SPIE’s International Symposium on Microelectronics and Micro-Electro-Mechanical Systems; 17.-19. December 2001, Adelaide, Australia.
8. T. Lindemann, W. Streule, G. Birkle, R. Zengerle, P. Koltay; “PipeJet – A simple disposable dispenser for the nanoliter range”; *to be published*, Proc. Actuator 2004; Bremen; Germany; 14-16 June, 2004.
9. P. Koltay, B. Birkenmeier, R. Steger, H. Sandmaier, R. Zengerle; „Massive Parallel Liquid Dispensing in the Nanoliter Range by Pneumatic Actuation”; Actuator 2002; 8th International Conference on New Actuators, 10-12 June 2002; Bremen; Germany, pp. 235-239.
10. P. Koltay, B. Bohl, S. Taoufik, R. Steger, S. Messner, H. Sandmaier, R. Zengerle; “Dispensing Well Plate (DWP): A highly integrated nanoliter dispensing system”; Proc. of IEEE-Transducers 2003; 8.-12.06.2003; Boston; USA; pp. 16-19.
11. R.M. Kaack, A. Jung, H.M. Wenz, R. Zengerle, M. Daub; “PCR-slide: A modular and cascable platform for DNA sample processing with integrated nanolitre dosage”; Proc. IEEE MEMS 2004, pp. 338-342.