

The Dispensing Well Plate: A Novel Device for Nanoliter Liquid Handling in Ultra High-Throughput Screening

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This article reports on a novel dispensing system for the massive parallel delivery of liquid volumes in the range of 50 nL. Due to the similarity of the device to conventional micro-well plates used for the storage of liquids, the device has been termed “dispensing well plate” (DWP). In contrast to other known micro dispensers, the DWP can consist of up to 1,536 dispensing units in parallel, all of which hold different reagents. The dispensing units can be arranged very closely at the pitch of conventional micro-well plates (2.25 mm or 4.5 mm). Driven by pneumatic actuation, a fixed volume of different liquids can be dispensed simultaneously and contact-free into micro-well plates or onto flat substrates. Because of this, the liquid handling in many chemical, biochemical, and pharmaceutical applications—especially within high-throughput screening (HTS)—can be sped up by a factor of 10 to 100. In our article, the basic operation principle of the device is presented, and experimental evidence of its extraordinary performance is given: a reproducibility of 2% to 5% and a homogeneity within individual droplet arrays of 1% to 2% has been measured, as well as viscosity independent performance for liquids in the range from 1 to 5 mPas. The applicability of the DWP technology within HTS is demonstrated by performing

a miniaturized kinase assay at 1 μ L assay volume in a 1536-well plate format. (JALA 2004;9:291–9)

INTRODUCTION

In modern drug discovery, potential drug candidates have to be identified from a huge number of different chemical compounds. This process is called high-throughput screening (HTS). It allows the testing of several 100,000 chemicals per day. In HTS, biochemical assays are performed in standardized containments termed micro-well plates. Today, well plates with 384 or 1536 wells at a pitch of 4.5 mm or 2.25 mm are handled within automated equipment. Around 1,000 dispensing cycles per minute are completed currently by conventional pipetting systems. To push the limit of HTS, assay volumes ranging from 2 μ L to 5 μ L have to be miniaturized even further, and the liquid handling has to be accelerated.^{1–4} Therefore, pipetting and dispensing systems are required, which can handle volumes in the nanoliter range and operate in a multi-parallel fashion compatible with well-plate formats. The DWP system presented in this article is able to speed up the liquid-handling process in HTS by a factor of 10 to 100, while at the same time reducing reagent consumption from the microliter to the nanoliter range.

SYSTEM DESCRIPTION

The complete DWP system consists of a pneumatic actuation unit driving a microfluidic chip termed

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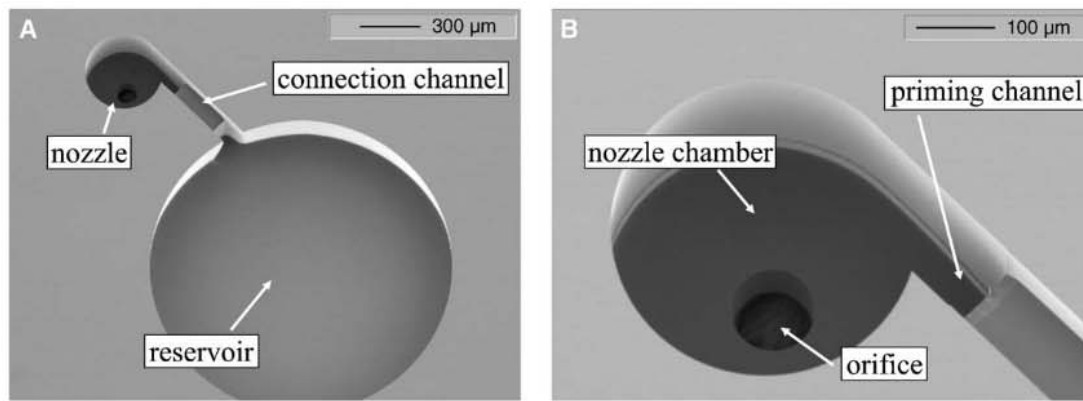


Figure 2. SEM micrograph of (A) a single dispensing unit of the DWP micromachined in silicon, and (B) a close-up of the nozzle chamber.

channels to the bottom of the reservoir and nozzle chamber, a 3-dimensional path building the connection channel was micromilled with a tapered single-lip cutter. In the final step, the lower nozzle with the nozzle orifice of the dispensing device was drilled with a diameter of 100 μm at 8,000 rpm. Figure 7 shows SEM pictures of a high-speed, micromilled dispensing unit.

Although all tests have been made using prototypes that have been cleaned by solvent or plasma treatment after use, the final version of the DWP will be a disposable device similar to a micro-well plate manufactured by replication technology like injection moulding.

APPLICATIONS

One key application for the DWP system within HTS is the rapid compound reformatting by the direct addition of nanoliter volumes to an assay. Therefore, the standard configuration for the DWP is an array of dispensing units where every nozzle is supplied from a different reservoir. This makes the DWP a flexible tool in which different liquids can be handled in neighboring wells as well. The dispensing performance of different liquids is in a certain range independent from liquid properties, because the liquid volume is predefined by the nozzle geometry. So, the

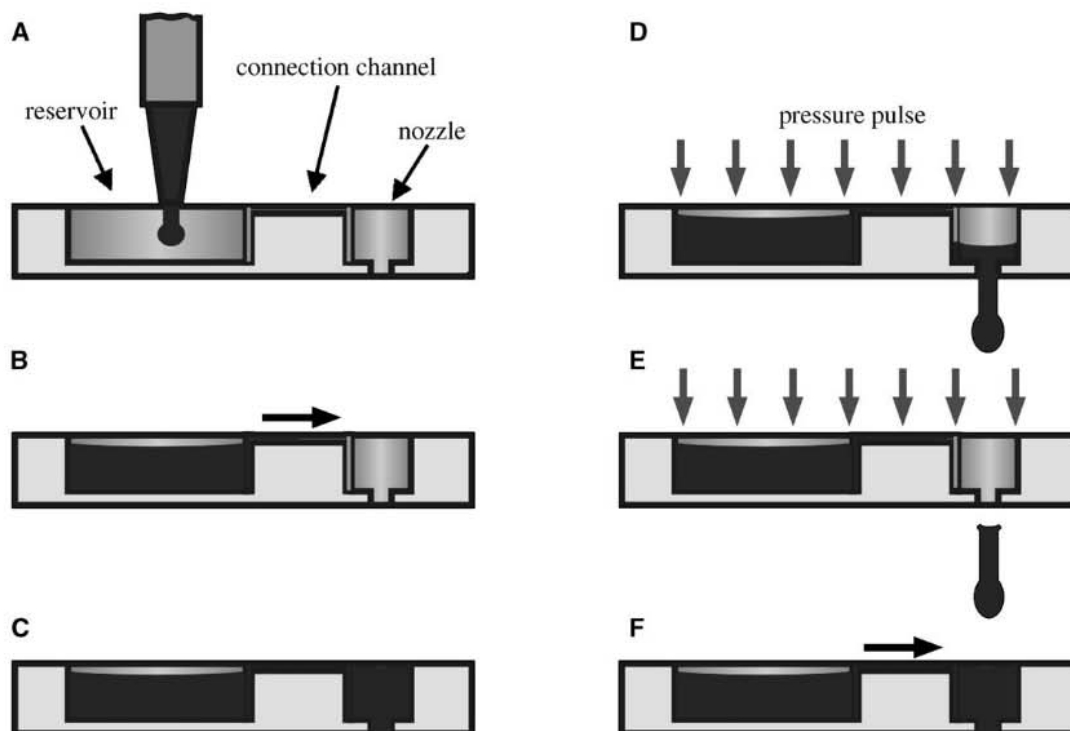


Figure 3. Schematic illustration of the DWP working principle: (A) Filling of the DWP, (B) capillary priming of the nozzle, (C) DWP ready for dispensing, (D) actuation of the DWP by pneumatic pressure, (E) complete depletion of the nozzle chamber during jet ejection, and (F) refilling by capillary forces.

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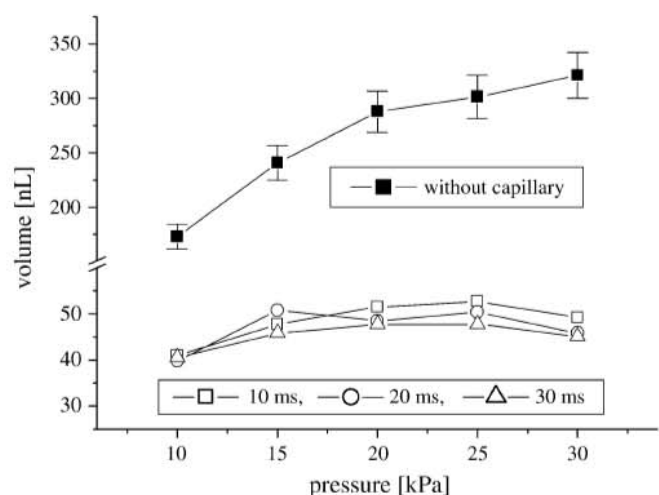


Figure 4. Dosage volume of dimethylsulfoxide (DMSO) as a function of pressure head and pulse duration. Compared are the values for the DWP prototype shown in Figure 5, A (hollow symbols) and a pressure-controlled dispenser concept consisting of a well with an orifice in the bottom without a capillary channel (full squares).

liquids can be arranged in a way that allows for assay arrangement.

One major advantage of the DWP system is small dispensing volumes, which can be handled accurately and in a highly parallel way. This is the ideal premise for applications in ultra-high throughput screening with assay volumes below 5 μL and extensive use of high-format 1536-well plates. High-format applications need highly parallel dispensing, especially when the simultaneous addition of liquids for time-dependent assays is necessary. The use of low-volume assays also allows the reduction of expensive reagents. This is also important for certain applications like protein crystallization for the realization of concentration gradients and for applications in SNP analysis.

Special, user-defined designs of the DWP system are also possible. In this way, different dispensing volumes can be combined on one DWP plate. This is important when working with concentration gradients.

A very special design of the DWP is referred to as a "bulk reagent dispenser." This DWP design is arranged in a way that all nozzles are supplied from a common reservoir. This can be realized easily by connecting all reservoirs of the standard configuration with additional capillary channels to each other. By filling liquid into any well, all dispensing units get filled by capillary forces and the same liquid can be dispensed through all units by the actuation pulse. A prototype of such a bulk reagent dispenser has been manufactured by high-speed micromilling of PMMA. The prototype is designed for dispensing of 384 times 1 μL aliquots of the same reagent simultaneously. Figure 8 shows the bulk reagent dispenser DWP and a top view of the plate filled with blue ink, so that all details of the connected structure are visible.

Further application of the DWP might go in the direction of using the DWP system as a storage device. The possibility of maintaining a certain assay arrangement or library over a longer period and dispensing defined nanoliter quantities from this arrangement, when necessary, offers new opportunities in liquid handling. However, intensive testing and validation is required to assure compound and dispensing quality over a longer period of storage.

EXPERIMENTAL CHARACTERIZATION

To drive the micromachined DWP chips, a prototype actuation unit has been constructed, which consists mainly of a pressure chamber above the DWP chip that can be pressurized by two pneumatic valves. A sketch of the system is shown in Figure 9. Figure 10 shows a dispensed array of DMSO in a micro-well plate. Using this actuation, unit experiments were carried out to characterize the dispensing performance of the various DWP prototypes.

GRAVIMETRIC MEASUREMENTS

The presented DWP prototypes have been intensively characterized by gravimetric measurements. The overall dispensed mass has been measured with a microbalance accounting for systematic errors due to evaporation or adsorption. From this data, the mean dosage volume per

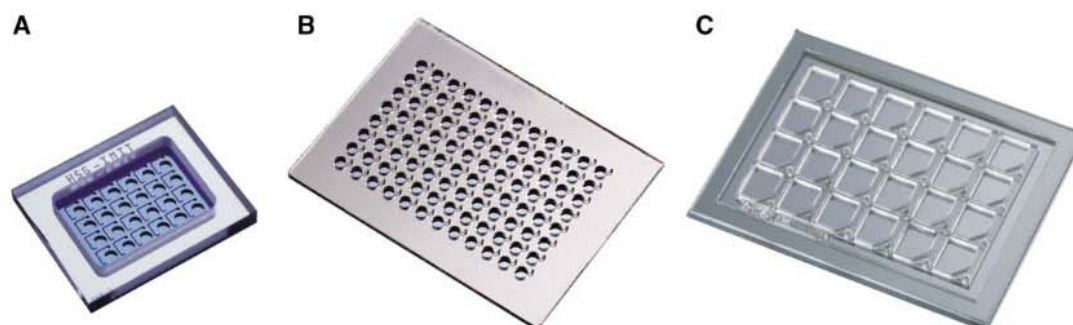


Figure 5. Fabricated DWP prototypes. (A) Silicon prototype with 24 dispensing units at a pitch of 2.25 mm, (B) Silicon/SU-8 prototype with 96 dispensing units at a pitch of 2.25 mm, and (C) Silicon/SU-8 prototype with 24 dispensing units at a pitch of 4.5 mm.



Figure 6. 384-unit DWP made by high-speed micromilling of COC.



Figure 8. Bulk reagent dispenser DWP with $384 \times 1 \mu\text{L}$ dispensing volume made by micromilling in PMMA.

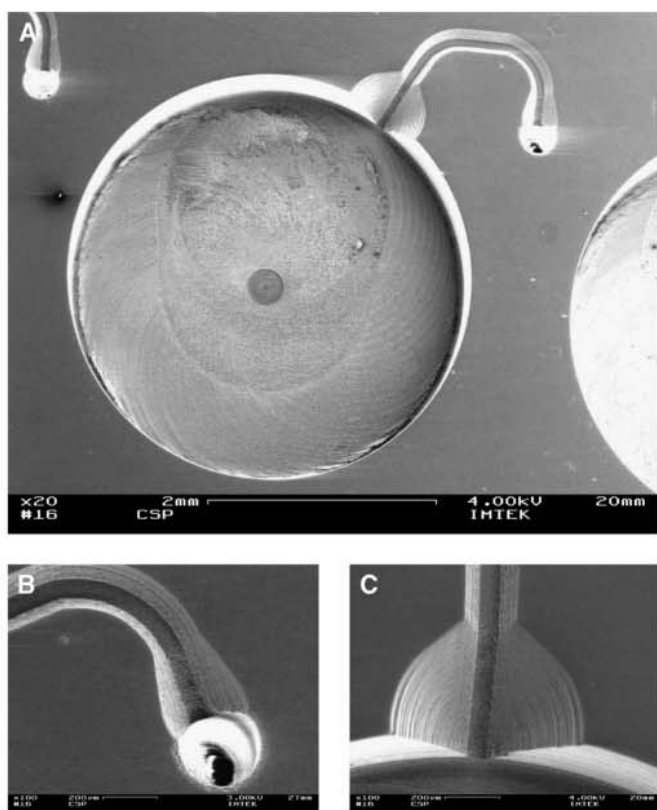


Figure 7. SEM picture of high-speed micromilled structures. (A) Dispensing unit with conical channel (upper/lower width: $200 \mu\text{m}/50 \mu\text{m}$), with a depth of $130 \mu\text{m}$; (B) detail of the connection from nozzle chamber to supply channel; and (C) detail of the connection from reservoir to supply channel.

channel has been calculated. In order to prove the working principle, the influence of pressure head and pulse duration on dosage volume was studied. The results of these experiments (Fig. 4) support the assumption that, essentially, the

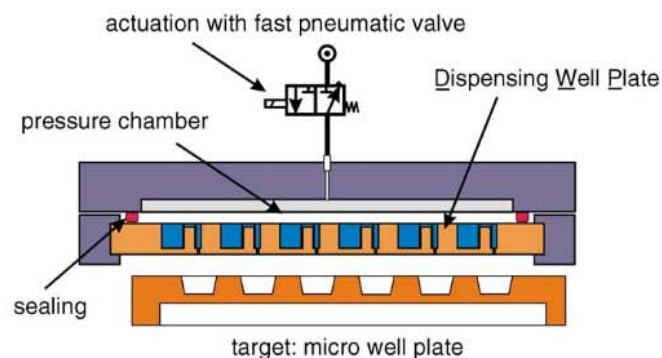


Figure 9. Schematic of the actuation unit.

volume contained in the nozzle channel is dispensed and no significant flow from the reservoir occurs during dispensing. No pressure dependence of the dosage volume has been detected, which proves the fixed-volume dispensing concept.

Using a solution of water with 0.5% surfactant (Nonidet p40 substitute; Fluka, Buchs, Switzerland) and DMSO as

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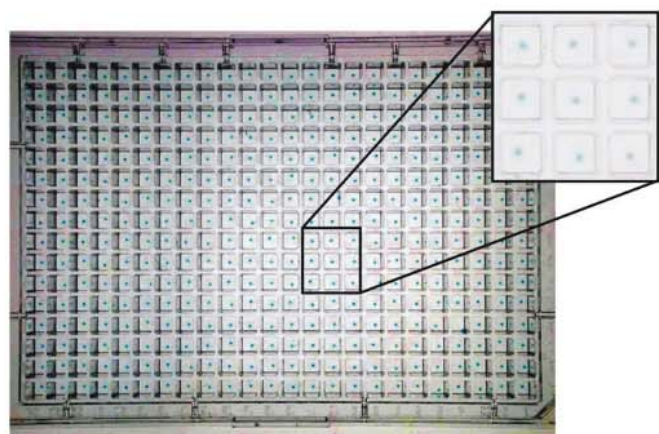


Figure 10. Array of 384, with approximately 50 nL dispensed DMSO droplets in the bottom of a micro-well plate.

dosage media, the reproducibility for the DWP prototypes made by silicon micromachining and SU-8 technology (Figure 5,b,c) was determined. The surfactant had to be added to the water because the contact angle of water on SU-8 is close to 90° , which hampers reliable self priming. By adding Nonidet, the wetness could be enhanced and a reliable priming of the nozzles could be achieved. The average dosage volumes per dispensing unit (Fig. 11), as a series of consecutive shots, show that identical volumes are obtained for different liquids. This observation supports the fixed-volume dispensing concept outlined before. Therefore, it can be expected that other solvents can be dispensed with similar accuracy also. A small influence on the surface tension, defining the liquid meniscus at the nozzle and on the upper side of the nozzle chamber, is probably evident but can be missed if comparing this with the total dispensing volume. The effect of surface tension and viscosity on the capillary filling is negligible if the refilling time is long enough to

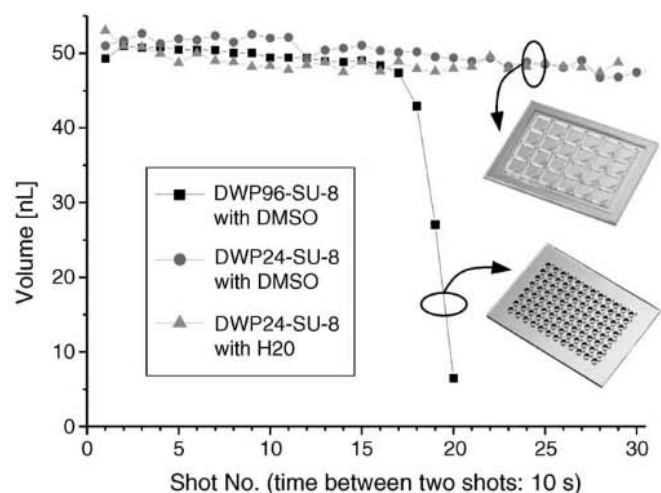


Figure 11. Reproducibility of the dispensing process for DMSO and water.

ensure proper refilling (ie, if successive dispenses are performed with a frequency of typically less than 5 Hz).

Furthermore, the experiments result in a good reproducibility of close to 3% in all cases. That and the robustness of the method discussed before constitute the basis for the excellent performance of the DWP. The main difference between the DWP prototypes with 24 dispensing units (384 format) and 96 dispensing units (1536 format), respectively, is that for the 1536 format, the reservoir depletion sets in much earlier. The reservoir volume of the 96-channel prototypes of about 800 nL is depleted within 16 shots, while the larger reservoirs of the 24-channel devices of more than 6 μL enable continuous dispensing. Therefore, one important goal for the further optimization of the 1536 format is to enhance the SU-8 thickness from 500 μm to more than 1000 μm to accommodate more liquid.

JET QUALITY

Based on the gravimetric experiments discussed, only statements regarding the average dosage volume per dispensing unit are possible. Stroboscopic imaging was applied to find out if the jet generation occurs homogeneously over all nozzles. Pictures of the orifice array have been taken at different times after starting a repeated dispensing process with a frequency of 1 Hz, which are shown in Figure 12. In the photographs, three neighboring orifices are visible in the focal plane. It is clear that the jet generation proceeds reproducibly and homogeneously. At a nominal pressure head of 20 kPa, the jets escape from the 100- μm wide nozzles at a speed of approximately 1.6 m/s and terminate in a spray of liquid after jet tear off. This is due to the fact that the density of air is much smaller than the density of the used liquids, which causes a high-speed airflow after jet tear off. Because of the Venturi effect, this airflow generates a spray that transports liquid out of the nozzle. How much liquid is transported through the spray is essentially determined by the nozzle shape as discussed in more detail in the article from Koltay et al.⁸ Although the spray effect degrades the jet quality, the dosage accuracy is hardly affected. Even the droplets generated on a flat substrate typically do not exhibit any satellites if the distance to the DWP during dispensing is small enough (approximately less than 1 mm). In this case, the narrow opening angle of the spray causes all sprayed droplets to rejoin the leading part of the liquid on the substrate. Another unintended side effect of the spraying is the formation of a corona of droplets around the orifices on the nozzle plate (Fig. 12). This is presumably due to the electrostatic charging of the droplets during the ejection process and might cause degradation of the surface properties or cross contamination during extended use of the DWP. Therefore, the reduction of the spray effect is a major target for further optimization.

BIO-CHIP READER MEASUREMENTS

In order to derive the statistics of the droplet distribution within a simultaneously dispensed droplet array, measurements

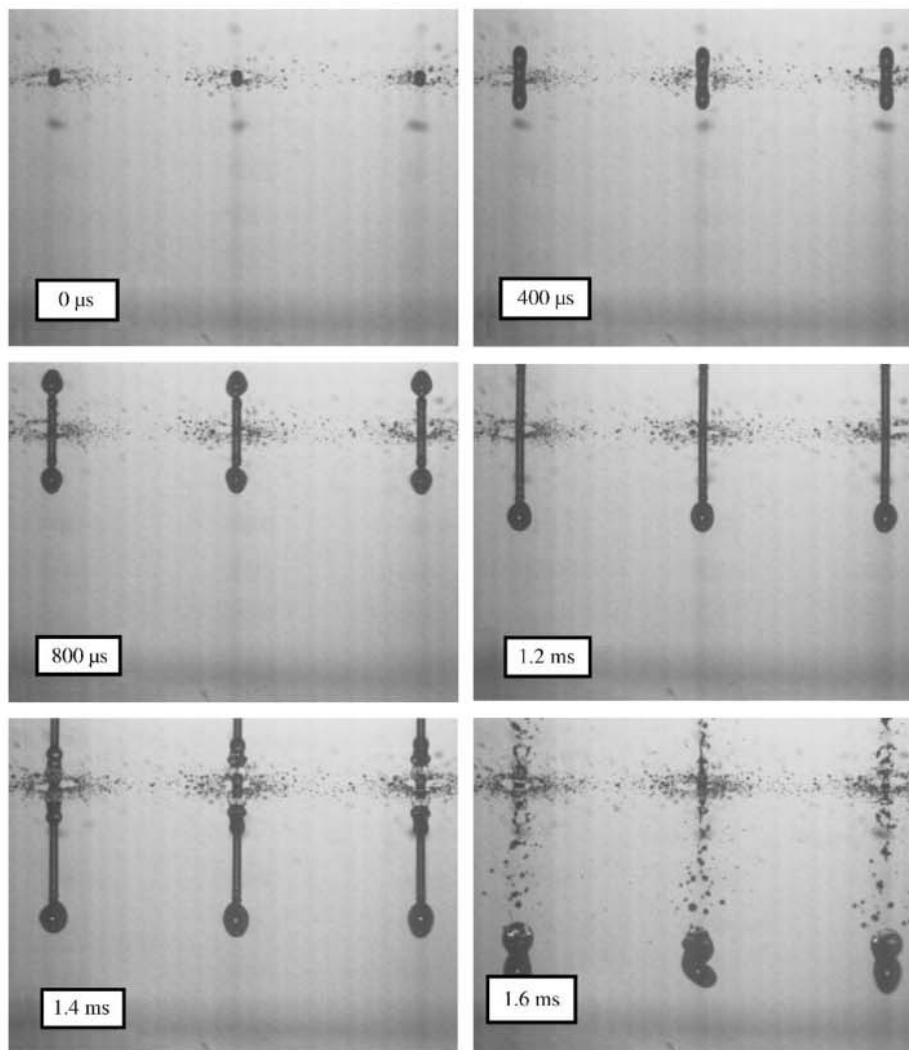


Figure 12. Stroboscopic images of the jet ejection from three nozzles of a DWP at successive points in time (dispensed medium: DMSO). The jets visible directly above the orifices are mirror images of the real jets moving downwards.

with a bio-chip reader (BioAnalyzer 4F; LaVision BioTec GmbH, Bielefeld, Germany) have been conducted. DMSO has been labeled with Dye630 (0.2 mol/L, emitting at 630 nm) and has been dispensed onto a glass slide using the DWP prototype displayed in Figure 5a. Because DMSO is hygroscopic, the droplets on the slide do not dry and the fluorescence signal is correlated very well with the droplet volume. This has been proven by a prior calibration. The calibration has been carried out by dispensing a series of volumes from 5 to 60 nL with a single channel NanoJet-type nanodispenser.⁹ The dispenser itself has been calibrated before by gravimetric measurements. Thus, the volume of the dispensed droplets was accurately known. Then, the same volumes were dispensed onto a glass slide and the fluorescence picture (Fig. 13a) was taken. By integrating the total signal over each spot and applying a linear fit, the slope of the calibration curve was to be 77873 ± 687 counts/nL.

Based on the calibration, the absolute volume of the individual droplets dispensed by a 24-channel DWP has been determined. The fluorescent image of the array spotted onto a glass slide is shown in Figure 13b. This shows that the homogeneity of the dispensing process and also the quality of the droplets is very good. An equal-signal distribution without satellite droplets can be observed. The quantitative analysis reveals that an excellent CV of 1% could be obtained. The determined mean volume of 42.9 ± 0.6 nL is slightly smaller than the values obtained by gravimetric measurements ($48 \text{ nL} \pm 3.5 \text{ nL}$; Fig. 4). The small systematic deviation of the measurements might be attributed to a slight drift of the camera signal over time. To avoid this source of systematic error, the calibration would be better performed on the same slide as the measurement (i.e., taking the pictures seen in Fig. 13a,b simultaneously). Compared with the gravimetric measurements, the fluorometric method provides

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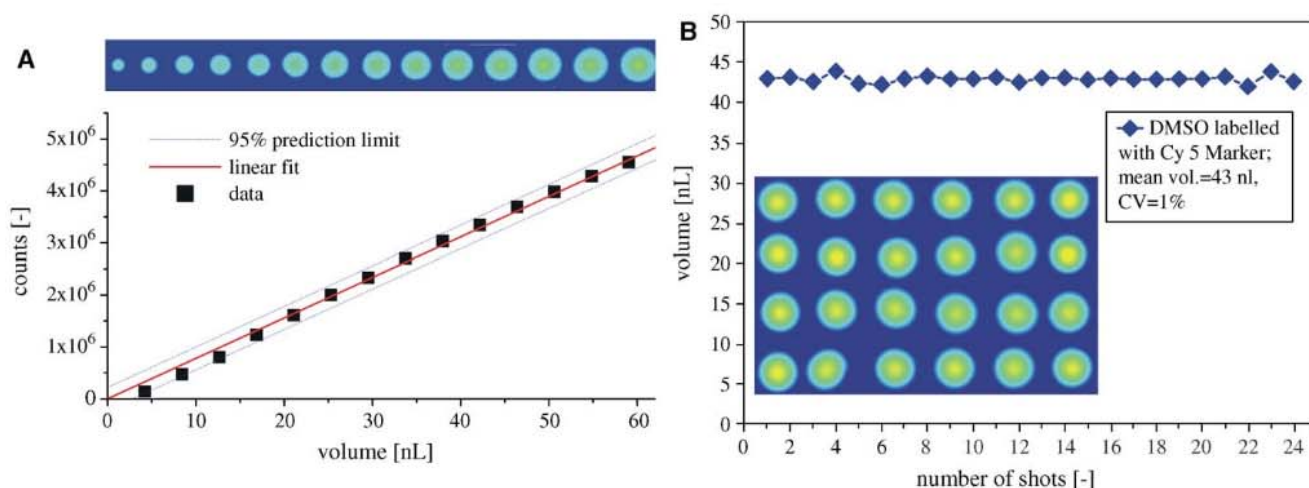


Figure 13. (A) Fluorometric image of a 5 nL to 60 nL droplet series and corresponding calibration line dispensed with NanoJet. (B) Fluorometric image of a droplet array dispensed by a DWP prototype and corresponding volumes per droplet.

more information and is much more accurate (relative error of 1.3%), but it is also more complicated. It is furthermore restricted to hygroscopic liquids; otherwise, a precise evaporation control would be required.

KINASE ASSAY

To demonstrate the possibilities of a highly parallel nanoliter dispenser in miniaturizing the assay handling in HTS, a kinase assay was prepared at a total assay volume of 1 μ L. This made it necessary to work in an ultra-low volume micro-well plate. For this reason, a 1536-well micro-well plate of Greiner was chosen, which had a maximum working volume of 10 μ L/well. Tests have shown that assay volumes of 1 μ L can only be read with the available standard plate reader equipment (Victor; Wallac, Turku, Finland²) with good quality of the measurements when using this type of well plate. The results of the miniaturized assay were compared to the reference case performed in a 384 well plate at an assay volume of 20 μ L.

The chosen assay is based on a non-fluorescent bisamide substrate (fluorescence is blocked by the peptide). It is

a commercially available substrate from Molecular Probes Europe BV (Leiden, The Netherlands).¹⁰ By cleavage of the peptide (trypsin), the bisamide is converted in a two-step process to Rhodamine 110. The Rhodamine 110 can be detected by fluorescence signals with an emission wavelength of 521 nm.

The assay formulation has been designed in a way that all liquids could be dispensed with a 384-unit DWP with a fixed-dispensing volume of 50 nL. For the reference case (20- μ L assay volume), a standard 384-well plate was used and prepared by hand pipetting. For both assays, the same end concentration of the liquids was chosen. The Rhodamine 110-based substrate had an end concentration of 100 μ M. The Trypsin was diluted to an end concentration of 0.05 U/mL in the assay. The protocol to prepare the plates is further detailed in Table 1.

Since the available DWP prototypes comprise only 384 dispensing units, the 1536 assay plate was not filled

Table 1. Assay formulation for the volumes 1 μ L and 20 μ L

		Miniaturized assay volume of 1 μ L		Assay of 20 μ L	
		Dispensed volume	Shots of DWP	Total volumes	
Buffer	HNPT 10% DMSO	50 nL	16	800 nL	16 μ L
Substrate	Rhodamine 110 substrate	50 nL	2	100 nL	2 μ L
Enzyme	Trypsin	50 nL	2	100 nL	2 μ L

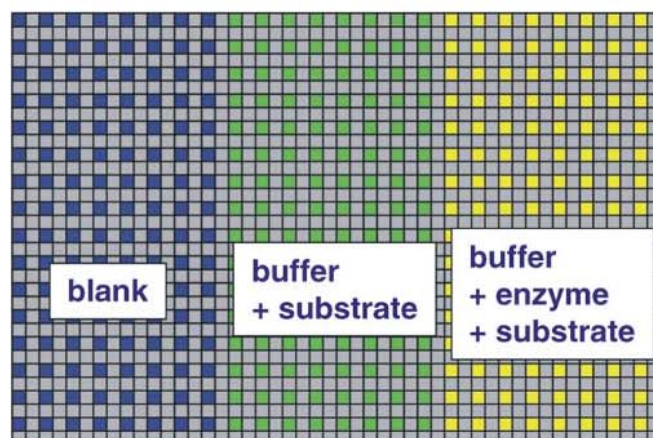


Figure 14. Arrangement of the assay components on the target well plate (1536 wells).

