FIXED VOLUME 384 CHANNEL NANOLITER DISPENSER FOR HIGHLY PARALLEL AND SIMULTANEOUS LIQUID TRANSFER INTO WELL PLATES

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Abstract

We present a highly parallel nanoliter dispenser on the footprint of a micro well plate (80 mm x 120 mm) (figure 1). The so called Dispensing Well Plate (DWP™) contains 384 individual dispensing units at a pitch of 4,5 mm and allows for transfer of liquid quantities of 50 nL to a receiving plate. The DWP™ is simply operated by a pneumatic pressure pulse. Tests with fluorescence measurements prove the CV of the dispensed volume to be better than 5 % for DMSO and/or aqueous solutions. Thus the device is able to increase dispensing speed as well as quality in High-Throughput-Screening (HTS) significantly.

Keywords: HTS, nanoliter dispenser, highly parallel, well plate, silicon micro machining

1. Introduction

Pharmaceutical research experiences an increasing need for highly parallel dispensing technologies in the nanoliter range to speed up drug development [1]. 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 1000 dispensing cycles per minute are accomplished currently by conventional pipetting systems. To push the limit of HTS, assay volumes ranging from 2 µL to 5 µL have to be miniaturized even further and the liquid handling has to be accelerated. Therefore we developed the DWP™ technology [2, 3] with the aim to improve this throughput significantly.

2. Design & Fabrication

The DWP™ contains a multitude of individual dispensing units, each consisting of three basic elements: a reservoir, a connection channel and a nozzle chamber (figure 2). The reservoirs can be filled with different reagents at volumes of up to 6.8 µL. Due to capillary forces the nozzle chambers are self priming via the connection channels. To drive the micromachined DWP™ a prototype actuation unit has been constructed, which consists mainly of a cavity (pressure chamber) that can be pressurized by a pneumatic valve. By applying a pressure pulse to the whole upper surface of the DWP™ a liquid volume defined by the dimensions of the nozzle is driven out generating a free flying jet ejected from each nozzle.

The proof of principle with a limited chip size of 1/16 of a full micro well plate and a smaller reservoir volume of 0,7 µL has been presented earlier [2, 3]. Now we present the full size plate with 384 parallel dispensing units as well as an increased reservoir volume of 6,8 µL enabling to validate the DWP™-technology in a real HTS environment. The technological challenge producing the DWP is met by applying 6" silicon wafer technology and deep reactive ion etching (DRIE). The processed microfluidic structures include nozzles with aspect ratios over 3 etched through the complete wafer thickness of 1 mm. A proper microfluidic layout made it necessary to use a multi-layer mask for the DRIE-process: a standard SIO2 layer, an aluminum layer with a high selectivity and a 20µm thick resist layer. The uniformity of all nozzles over the complete wafer is important for the dispensing quality and homogeneity of the system.

3. Dispensing Quality and Application

The presented DWP prototype has been intensively characterized by gravimetric and fluorimetric measurements. Fluorescence measurements performed with a Wallac/Victor² plate reader and Europium 3.5 nM in DMSO (2 µL = 40 dispensing cycles) show that the homogeneity over all 384 dispensing units is within a CV of 5% (figure 3).

The 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 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 (figure 4) prove the robust and reliable performance. Using water and DMSO as dosage media the reproducibility for the DWP prototypes displayed in figure 4 has been determined with a CV of about 1% and better.

To demonstrate the potential of a highly parallel nanoliter dispenser in miniaturizing the assay technology within HTS, we prepared a kinase assay based on Rhodamine 110 at a total assay volume of 1 µL. This miniaturized assay has been compared to a reference case with equal end concentrations at an assay volume of 20 µL. Comparison of the measured signal counts of the 1 µL and the 20 µL assay reveals that although the 1 µL assay is at the low end of the plate reader capability the signal variation with a measured CV of 7.9% is comparable to that of the hand pipetted 20 µL volume with a CV of 7.3%. The relative dispensing quality therefore has to be in the nanoliter range as accurate as for the larger assay volume. Taking into account that the assay was performed in different plate sizes (1536 for the miniaturized and 384 for the reference case) this demonstrates the reduction of the assay volumes by a factor of 20 without loosing quality of the measurement results.

4. Conclusions

The full-size 384 DWP-Dispenser for massive parallel dispensing of liquids in the nanoliter range has been presented. The dispensing performance was successfully characterized by gravimetric and fluorimetric experiments. The proposed DWP method was proven to be very accurate, scalable, simple and robust. The DWP-technology therefore has the potential to speed up the liquid handling in HTS by a factor of 10 – 100 and to reduce reagent consumption by a factor of 10-20 in the future. The applicability of the DWP method in a HTS laboratory environment has been demonstrated by performing a miniaturized kinase assay with comparable quality to conventional assay volumes. In case of the demonstrated miniaturized assay the reagent consumption could be reduced by a factor of 20.

Acknowledgements

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References

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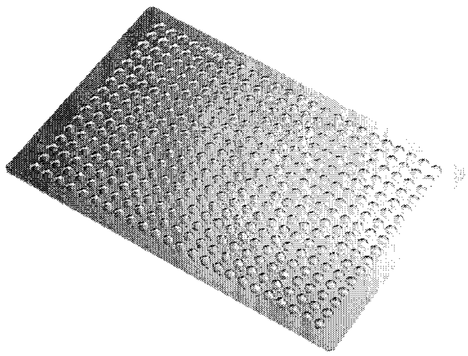

Figure 1. Photograph of a 384-unit DWP chip made of a 1 mm thick silicon wafer (pitch: 4.5 mm).

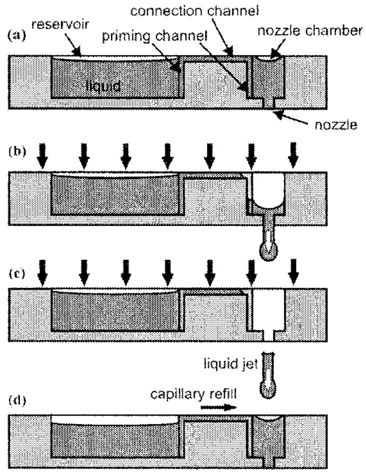

Figure 2. DWP dispensing process:
a) basic elements
b) jet ejection by pneumatic actuation
c) dispensed volume defined by the volume of the nozzle chamber
d) refilling by capillary forces

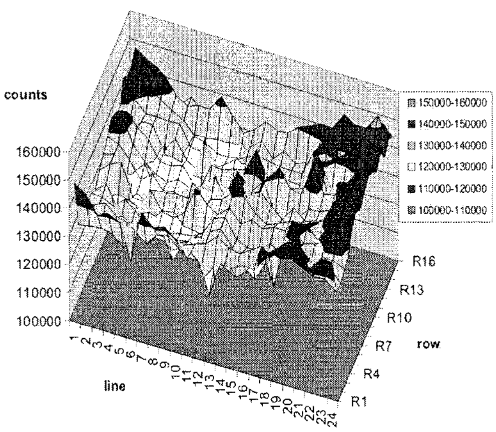

Figure 3. Diagram of a fluorescence measurement of a dispensed 384 droplet array. (measured with a Wallac/Victor² plate reader / DMSO with 3.5nM Europium 40 dispensing cycles). Measurement has a CV of 4.9 %.

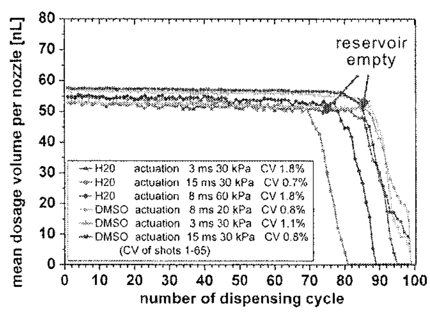

Figure 4. Gravimetrical measurement of the mean dosage volume of a section of 24 nozzles over successive dispensing with H2O and DMSO with different pressure pulse parameters.