# DROP IN DROP NANOLITER KINASE ASSAY MADE WITH HOT EMBOSSED DISPOSABLE MULTI CHANNEL DISPENSER

**R. Steger[1], C. Mehne[2], N. Wangler[1], M. Heckele[3], R. Zengerle[1] and P. Koltay[1]**

[1]Laboratory for MEMS Applications, IMTEK, University of Freiburg,
[2] Institute of Microstructure Technology (IMT), University of Karlsruhe,
[3] Institute of Microstructure Technology (IMT), Forschungszentrum Karlsruhe,
all Germany

**Abstract**

We present a new disposable "Dispensing Well Plate" (DWP$^{TM}$) manufactured by hot embossing and its application for compound storage and processing of nanoliter assays by drop in drop technique. The DWP-dispensers have been manufactured by a new low cost process creating the 100 µm nozzles directly within the molding process. Fluorescence measurements indicate a good homogeneity of the dispensed volumes better than 5 %. The DWP$^{TM}$ was used to perform a kinase assay based on 100µM Rhodamine substrate with a total assay volume as low as 200 nL. The assay was arranged in a checker board structure on conventional microscope slides. Fluorescence read out showed an good quality of the reaction signals with a robust z-value of 0.57.

**keywords: microfluidic, nanoliter dispenser, miniaturized assays, high-throughput-screening, HTS**

## 1. Introduction

There is a strong demand within the pharmaceutical und biochemical industry to create an increasing amount of data points within the high throughput screening in drug discovery [1]. On the other hand the costs of compound libraries and liquid handling have to be kept constant or should be reduced. One way to establish this is to reduce the assay volumes in the sub-µL range. Some research groups established solution phase based assays on slides or special nano-well plates [2, 3, 4]. One challenge within these developments is the precise and parallel handling of nanoliter volumes. The microfluidic DWP$^{TM}$ dispenser [5] is an appropriate tool for this task, which can be used as direct interface between the conventional storage in micro plates and the assay handling on slides and nanoplates.

## 2. Manufacturing process

The DWP-technology is characterized by arraying microfluidic dispensing units consisting of a reservoir, a capillary connection channel and a nozzle (fig.1). The pneumatically dispensed volume is defined precisely by the geometry of the nozzle and is greatly independent from actuation parameters. To manufacture the DWP$^{TM}$ we developed a hot embossing process using COC polymer. The mold insert was manufactured by high speed micromilling of steel (fig.2). By the hot embossing process

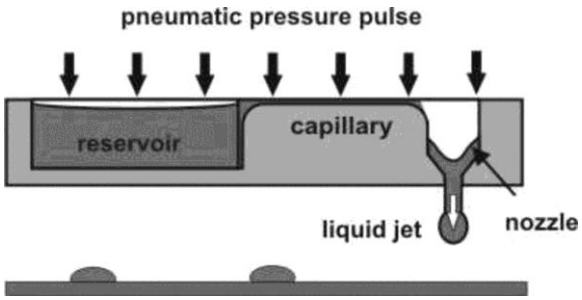


**Fig. 1** *Basis elements of the DWP™ dispensing process (schematic): reservoir, capillary and nozzle. Jet ejection occures by pneumatic actuation and refilling by capillary forces when pneumatic pressure turned off.*

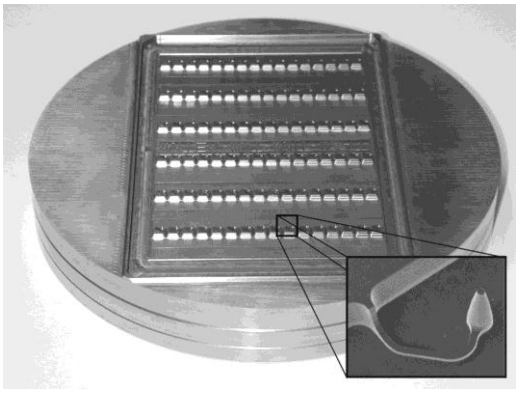

**Fig. 2** *Mould insert for a 96 channel DWP in the format of a micro well plate (127.7mm x 86,5 mm) with aspect ratios of 3 made by high speed micromilling of steel*

the arrayed dispenser structures have been successfully replicated on the format of a microtiter well plate (fig.3) and in smaller 24-well format (35 mm x 26 mm). The nozzle orifices have been directly molded with a diameter of 100 µm with very good quality for contact less dispensing (fig.4).

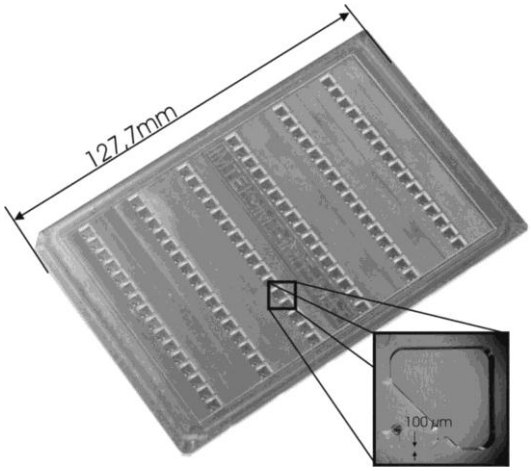


**Fig. 3** *Hot embossed 96 channel DWP in the format of a micro well plate (127.7mm x 86,5 mm) made from PMMA and COC with nozzles molded directly*

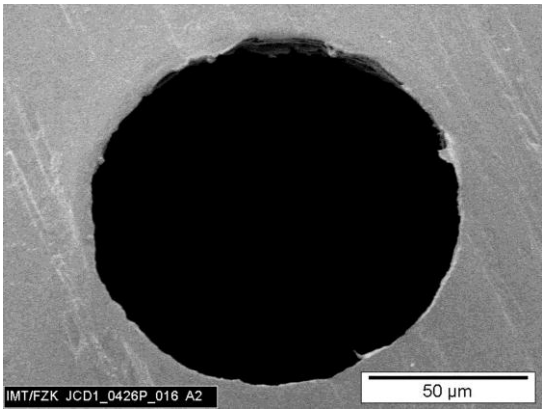


**Fig. 4** *DWP nozzle orifice made by direct embossing of through holes with a diameter of 100 µm. Sharp edged quality is important for good dispensing quality*

## 3. Application test:

The hot embossed DWP™ plates have been used for storage tests. DMSO- and water-based buffers were filled in the reservoirs and dispensing quality was tested. Afterwards the filled DWP was stored at temperatures of –20°C several days. Tests showed no loss of the dispensing quality directly after defrosting (fig. 5). Hot embossed 24-channel DWP™s have been used to perform a 200 nL kinase endpoint assay in a checker board

structure directly on a slide. Therefore 100 nL of HNPT-buffer and Trypsin in concentration of 200 U/ml were dispensed. Into these droplets either 100 nL of 100 µM Rhodamine 110 substrate or pure buffer were dispensed in a checker board manner. The fluorescence read out showed an excellent signal response with no cross contamination (fig.6). In the pharmaceutical screening community a statistical parameter the Z-value [6] is used to evaluate and validate performance of assays. The Z-value for the checker board signals showed a robust value of 0.57 (1 from 6 measurements all with Z' >0,5).

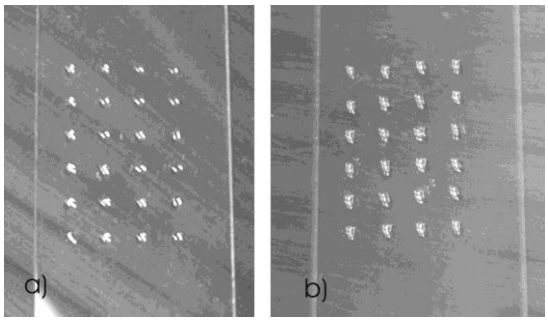


**Fig. 5** *4 x 6 droplet array with DMSO Buffer dispensed with hot embossed DWP a) before storage and b) after storage at –20°C directly after defrosting*

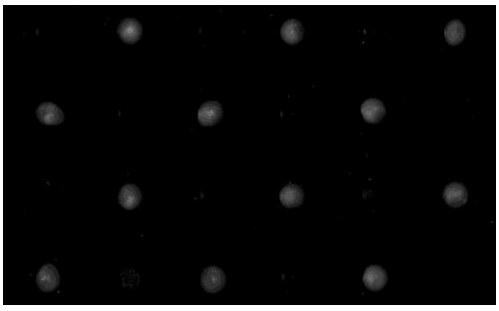

**Fig. 6** *Fluorescence read out of Kinase Assay with Rhodamine 110 substrate printed with the DWP in a checker board structure drop in drop with the enzymatic trypsin buffer (total volume 200 nL).*

## 4. Conclusion

It was demonstrated that disposable plastic DWP$^{TM}$ made by hot embossing can be used for highly parallel dispensing of nanoliter volumes. With the disposable DWP$^{TM}$ plates miniaturized assays with a total volume of 200 nL consisting of 3 different components have been realized. The measured assay quality with a Z-value of 0.57 proofed a good dispensing quality. Therefore the DWP$^{TM}$ nanoliter dispenser is an interesting tool in creating higher throughput with low liquid volumes in pharmaceutical ultra-high-throughput-screening.


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