I-DOT One & the Droplet Microarray (DMA)

Miniaturized cell-based screening on demand using Aquarray´s Droplet Microarray in combination with the I-DOT One non-contact low-volume dispensing platform

v1.0
application note

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We, the Dispendix GmbH, are a spin-off of the Fraunhofer Institute for Manufacturing Engineering and Automation (IPA) in Stuttgart, Germany. At IPA a novel liquid handling technology, called I-DOT („Immediate-Drop-On-Demand technology“) has been developed to establish an efficient, flexible alternative non-contact solution for liquid handling tasks for nano- to microliter volumes.

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Abstract

Cell-based screenings are essential for identification and optimization of pharmaceutically active substances. Aquarray GmbH (Eggenstein-Leopoldshafen, Germany) develops products for miniaturized cell screening applications based on its proprietary Droplet Microarray (DMA) technology. The combination of DMA technology with the I-DOT One (Dispendix GmbH, Stuttgart, Germany), a non-contact low volume dispensing system, enables screening on low cell numbers (1-200 cells per experiment) in nanoliter droplets, therefore, opening new perspectives for screenings of stem cells, iPSC and primary cells for more predictive and physiologically relevant data.

The I-DOT One allows dispensing of live cells, as well as compounds and reagents down to nanoliter volumes in precise locations, which is a prerequisite for robust and reproducible screening on the DMA platform. In this application note, we present a methodology for dispensing different liquid volumes and cell numbers using the I-DOT One into individual nanoliter droplets formed on DMA slides.

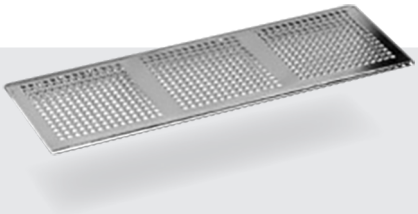

Figure 1: Droplet Microarray, DMA (Aquarray GmbH (Eggenstein-Leopoldshafen, Germany)

Figure 2: I-DOT One MC, low-volume non-contact dispensing device

Introduction

Droplet Microarrays (DMA, Figure 1) are used to perform high-throughput screening of live cells with various chemical libraries in a miniaturized format. Cells can be cultured and treated in droplets of nanoliter volumes and analyzed using conventional read-out assays involving fixation and staining followed by fluorescence microscopy.

The core of the DMA-technology is a transparent array of hydrophilic (extremely wettable) spots separated by superhydrophobic (extremely water repellent) barriers. The extreme difference in wettability allows for droplets of aqueous solutions to form and stay stable on hydrophilic spots without a need for physical barriers.

The "Droplet Microarray Plate One" consists of a transparent array of hydrophilic spots ("wells") incorporated into a frame in the standard SBS/ANSI format and is compatible with the I-DOT One MC dispensing system. Each spot is capable of trapping volumes from 50 to 250 nL. A diverse set of standard array geometries and spot sizes is available.

The I-DOT One MC (Figure 2) is a low-volume non-contact dispensing system. The I-DOT system delivers discrete droplets in the nanoliter range, i.e. the dispensing range starts in the low nanoliter, but can also dispense several microliter in seconds.

To retain the cells in good condition it is necessary that evaporation is reduced to a minimum. A humidity control can be attached to the I-DOT. To minimize evaporation during dispensing the system can be optionally equipped with a humidity control to reduce the evaporation of the tiny cell culture volumes and keep the cells in a good condition.

Materials and Methods

For the dispensing of water and cells an I-DOT One MC dispensing system equipped with a humidity control was used. Humidity was set to 70%. I-DOT PURE Wells (90 µm orifice, droplet volume between 15 and 45 nL per droplet) were filled with 80 µL of water or cell suspension. A Droplet Microarray was inserted into the I-DOT One MC. Water was dispensed in volumes of 50, 100, 150, 200 and 250 nL; cells were dispensed in volumes of 100 nL.

Prior to dispensing Jurkat cells were counted and diluted to the desired cell concentration in cell culture medium. HeLa cells were trypsinized according to a standard protocol, counted and diluted to the desired cell concentration in cell culture medium. Cells were diluted to final concentrations of 1*10^6 and 1.5*10^6 cells/mL for obtaining 100 and 150 cells per spot in 100 nL, respectively. Cells were stained with Calcein AM for visualization by placing 3 mL of both cell suspensions in sterile falcon tubes. After adding 1.5 µL of Calcein AM solution (1 mg/mL), cells were incubated for

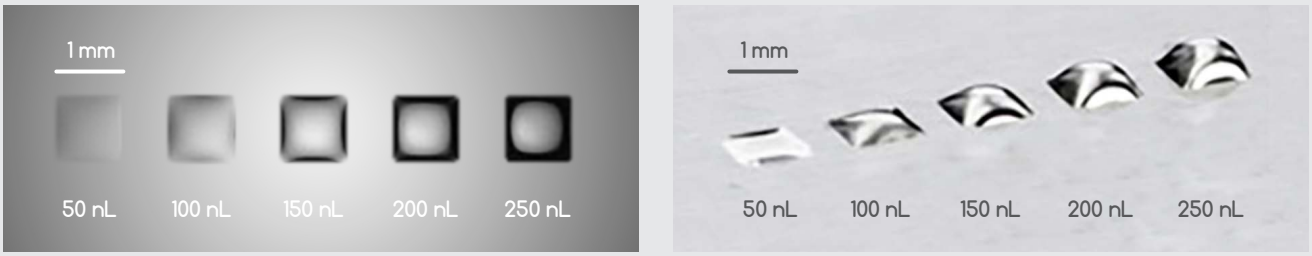

Figure 3 and **4:** Dispensing of defined volumes (water from 10 to 250 nL) into selected hydrophilic spots on the Droplet Microarray

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15 minutes in a cell culture incubator prior to dispensing. After dispensing an array with water was imaged (Figure 3 left and Figure 4 right, respectively). Cells were imaged using Keyence BZ-9000 and Olympus IX81 microscopes. Cell number was estimated by counting Calcein-positive cells using ImageJ software.

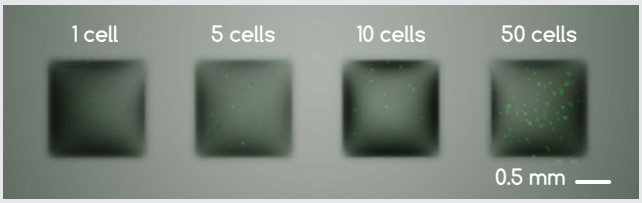
Results

By using the I-DOT One dispensing system the DMAs were successfully dispensed with distinct volumes of water ranging from 50 to 250 nL in precise locations on the DMA (Figure 6). No cross-contamination between spots during dispensing was observed.

Jurkat and HeLa cells with 100 and 150 cells per spot were dispensed on a full array field consisting of 169 spots. Dispensing time was 80 seconds. The droplets across the whole field were homogeneous in size and no evaporation during dispensing was observed. Cell number per spot was 100 or 150 cells cells on average, as expected, and had standard Poisson distribution (Figure 8) since the I-DOT does not count the cells but dispenses precise volumes.

Different amounts of cells per spot were dispensed. For this, different dilution of cells with the desired final concentrations were prepared and cells in a volume of 100 nL per spot were dispensed. As demonstrated in Figure 7, the number of cells per spot was evaluated to be very close or identical to calculated number of cells.

The viability and morphology of Jurkat and HeLa cells was monitored 24 hours after dispensing, showing viability values of both cell types of over 95%. As expected in conventional cell culture platforms, HeLa cells had normal spread morphology.



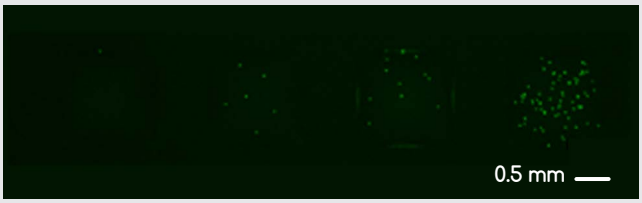


Figure 6 (left) **and 7** (right): After dispensing cells were analyzed by microscopy for the number of cells dispensed, cross contamination (Figure 6) and viability of cells after dispensing (Figure 7).

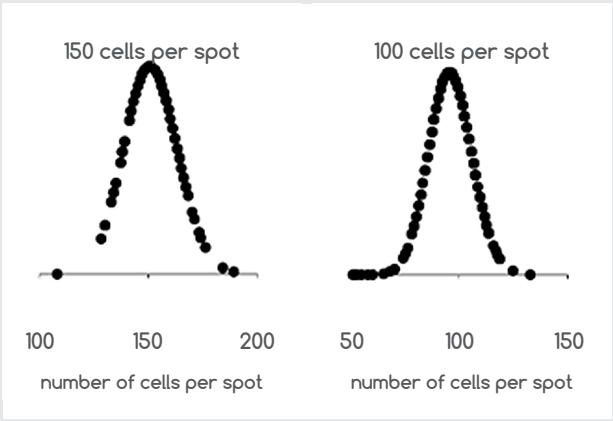


Figure 8: Defined number of cells can be dispensed using the I-DOT One system

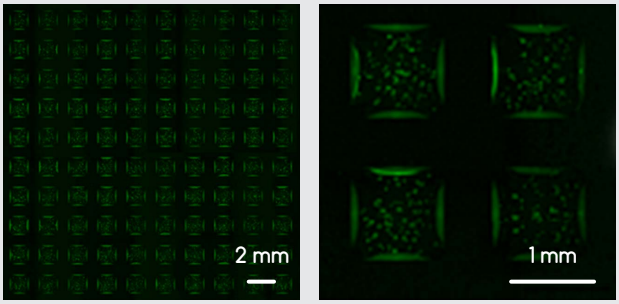


Figure 9: Due to the dispensing run which is finished in seconds and the humidity control, a high viability and a normal spread morphology of the cells can be observed after 24 h incubation.

Conclusion

In this application note we presented initial experiments using the I-DOT One low volume dispensing system and Droplet Microarray (DMA) platform. We demonstrated that using the I-DOT One precise and controlled volumes could be dispensed in distinct locations on the DMA without cross-contamination between spots. We were able to dispense live cells (Jurkat and HeLa cell lines) obtaining controlled numbers of cells per spot. We could dispense an array of 169 spots within 80 seconds without observing any evaporation during dispensing. Viability and morphology of cells cultured on this array for 24 hours were comparable to conventional cell culturing platforms. Taken together, the combination of the I-DOT One low volume dispensing system and Droplet Microarray (DMA) platform allows to perform many kinds of cell-based assays in an automated and miniaturized manner, where cells, compounds or reagents can be dispensed in distinct droplets. Such a system enables physiologically relevant screenings using minute amounts of primary and stem cells and opens new possibilities for fundamental research, drug discovery and healthcare.

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