Technical Note

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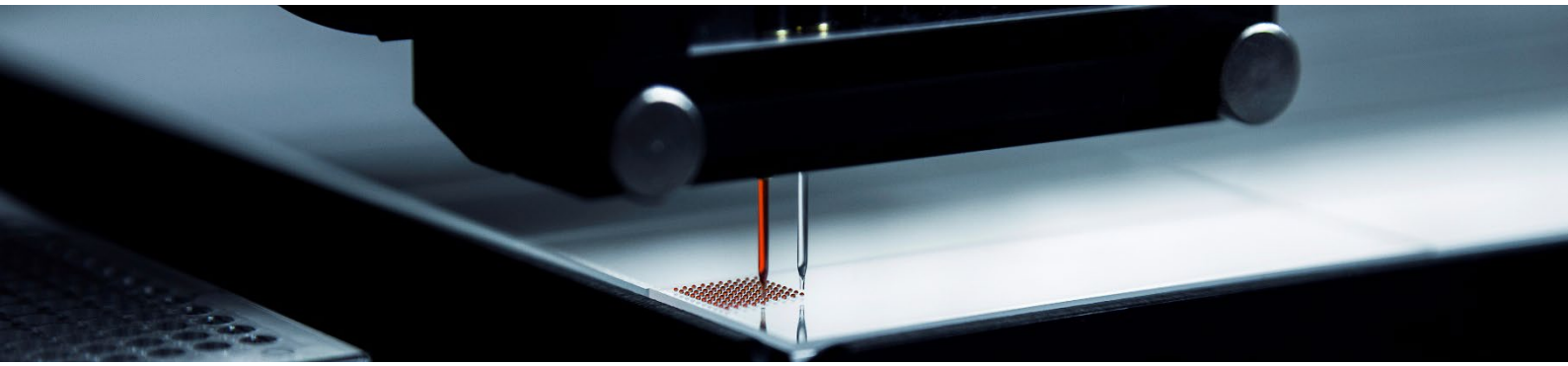

sciPULSE LOW VOLUME
Dispensing of small drop volumes in the range of 150 - 220 pL

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Abstract

SCIENION's sciFLEXARRAYER platforms are equipped with Piezoelectric Dispense Capillaries (PDCs) that enable dispensing of low volumes for miniaturized applications such as microarrays or biochips.
Here we show that the sciFLEXARRAYER plug-in solution sciPULSE LOW VOLUME, a hardware device combined with a specific software feature, enables the generation of droplets between 150 pL and 220 pL to reduce dispensed volumes or decrease spot diameters with SCIENION's PDC 80. This is of special interest for miniaturization and multiplexing of applications to optimize parameters such as sensitivity and speed.

Introduction

SCIENION's high-precision contact free printing technology, sciDROP PICO, is based on piezo-driven pulses on an inert glass capillary for accurate and precise droplet deposition under various conditions. When an electrical voltage is applied on piezoelectric materials, the electrical energy is converted into mechanical energy. Equipped with Piezoelectric Dispense Capillaries (PDCs), the generated mechanical energy is transferred to the fluid inside the PDC as a defined wave function. This results in a pressure wave that oscillates in the fluid followed by interference and a drop will be displayed out of the PDC orifice.

In principle, sciDROP PICO enables low volume dispensing ranging from 10 pL to 800 pL per drop for miniaturized applications that require reduced drop volumes or small spot diameters. The size and shape of generated droplets using SCIENION's sciFLEXARRAYER instruments depends on many different factors such as the size of the integrated PDCs. Furthermore, the individual parameters of each PDC, such as

voltage, pulse and frequency, need to be identified and adjusted in the sciFLEX software of the instrument to enable the most suitable drop generation for each specific application. Besides the PDC parameters, the stability of the droplet is also influenced by

the fluid properties such as viscosity, surface tension or density. The correct adjustment of the spotting parameters of different sample types is often time-consuming and requires some experimental experience. The implementation of protocols and workflows is therefore associated with high development costs. The utilization of a sciPULSE device allows the use of standardized application packages in combination with a specific software that enables changing the setup of the standard sciFLEX software feature for dispensing of challenging samples as well as reduction of drop volumes.

Here we show that sciPULSE LOW VOLUME software feature facilitates the adjustment of the optimal PDC parameters such as the applied pulse shape, voltage, dwell time and frequency for the generation of droplets between 150 – 220 pL with the PDC 80. Importantly, the sciPULSE LOW VOLUME can be used for miniaturized applications to reduce droplet volumes and spot sizes, as it generates droplets as little as 150 pL and spot diameters below 130 µm with the PDC 80 that normally interacts in the volume range of 360 – 440 pL.

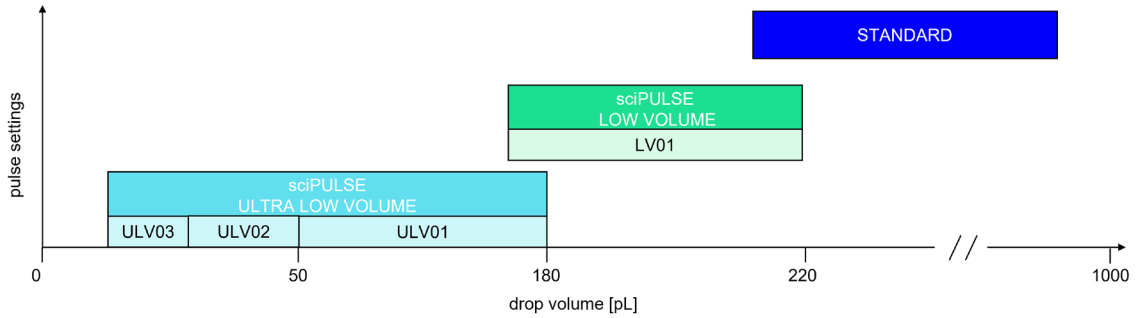

Figure 1 Overview of achievable drop volumes with standard pulse, sciPULSE LOW VOLUME and sciPULSE ULTRA LOW VOLUME pulse. The sciFLEX software can be operated with standard settings (dark blue) and lead to the generation of droplets between 200 – 800* pL. The sciPULSE LOW VOLUME (green) including the LOW VOLUME (LV01) pulse facilitates dispensing below 220 pL. The sciPULSE ULTRA LOW VOLUME application package (light blue) enables the generation of droplets in the volume range of 18 - 180 pL depending on the used pulse shape ULTRA LOW VOLUME (ULV01-03). ***Please note** that exact achievable drop volumes depend on many parameters, such as PDC size, sample type and configuration.

Materials and methods

Drop formation with standard pulse

SCIENION offers a range of PDCs with various sizes to achieve certain drop volumes that are shown in Table 1. Typical drop volumes range from 300 pL to 500 pL, which lead to spot diameters of 130 µm to 250 µm depending on the surface wettability of the substrates and the spotting samples. Due to the increasing interest in miniaturization and multiplexing of applications, smaller sample volumes are required, either to reduce dispensed materials or spot diameter. This can be facilitated by the integration of the sciPULSE LOW VOLUME application package in the sciFLEXARRAYER platforms.

Table 1. PDC sizes and the correlating drop volume range.

Size	Volume range [pL]
PDC 60	220 - 300
PDC 70	300 - 360
PDC 80	360 - 440
PDC 90	440 - 520

Applying sciPULSE LOW VOLUME

With regular drop volumes of approximately 400 pL using the PDC 80, there are limitations to achieve narrow arrays or small spot diameters. The sciPULSE LOW VOLUME allows the generation of low volume droplets between 150 – 220 pL with SCIENION's medium sized PDCs, such as the PDC 80.

The setup of the PDC consists of a few easy steps:

1) In the Nozzle Setup Tab of the sciFLEX software, the sciPULSE button is found
2) The pulse list opens by clicking the sciPULSE button
3) Select "sciPULSE_LV01" and press send
4) Increase initial Voltage [V] by 25% ± 5% to achieve desired low drop volume

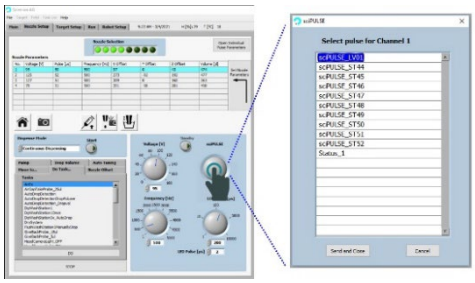

Figure 2. Nozzle Setup tab of the sciFLEX software and pulse list

Detailed information can be found in the sciPULSE LOW VOLUME quick start manual.

Results and discussion

First, the drop volume of the PDC 80, that is generally in the range of 360 – 440 pL (Table 1) was reduced by loading the pulse shape sciPULSE_LV01 and setting up the PDC as described above. Depending on the applied voltage in the Nozzle setup, the same PDC is able to print in a wide drop volume range. The sciFLEX software analyzes the drop images for straight flight path as well as for drop volume. In Figure 2 it is shown that by changing the voltage from 107 volts to 87 volts any low volume droplet between 150 pL and 200 pL can be generated. In conclusion, by using the sciPULSE LOW VOLUME, drop volumes can be decreased up to 65%.

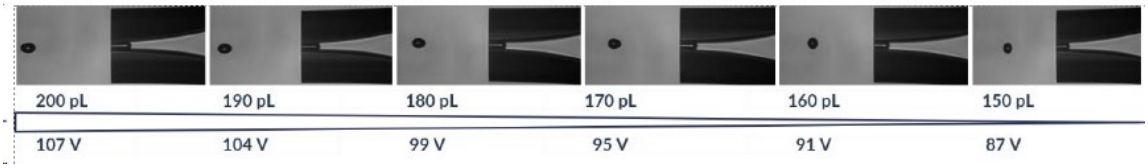

Figure 2 Adjusting the droplet of a PDC 80 using sciPULSE LOW VOLUME. By loading the pulse and adjusting the voltage, drop volumes ranging from 150 pL - 200 pL can be achieved.

Aside from the reduction of total printed material, miniaturization may also require the deposition of reagents onto very small areas such as for printing onto defined sensor structures, covering sensing electrodes or filling microwells. Here, a small drop volume and respectively a small spot diameter is of advantage. Applying sciPULSE LOW VOLUME allows arrays to be much narrower. Due to the reduced drop volumes also the spot diameter can be reduced significantly. Therefore, 1 mg/ ml Cytochrom C from bovine heart (Sigma Aldrich) in PBS was printed in 20x20 arrays on a sciCHIP H2 (SCIENION) with a spot pitch of 150 µm under the same experimental conditions and both PDC settings, the standard pulse shape and the sciPULSE LOW VOLUME setup (Figure 3A). As expected, PDC 80 with standard pulse settings generated droplets of 400 pL, whereas the application of the sciPULSE LOW VOLUME setting reduced the drop volume by more than 50 % to 190 pL. Subsequently, each spot and their correct positioning as well as the spot diameter were evaluated using the online QC analysis of the software. The standard settings of the PDC 80 could not be used to print arrays with 150 µm spot pitch as in this case the spots merge and defined spot sizes cannot be determined. Applying the sciPULSE LOW VOLUME feature, 20x20 arrays can be printed with spot diameters as low as 70 µm on a hydrophobic surface.

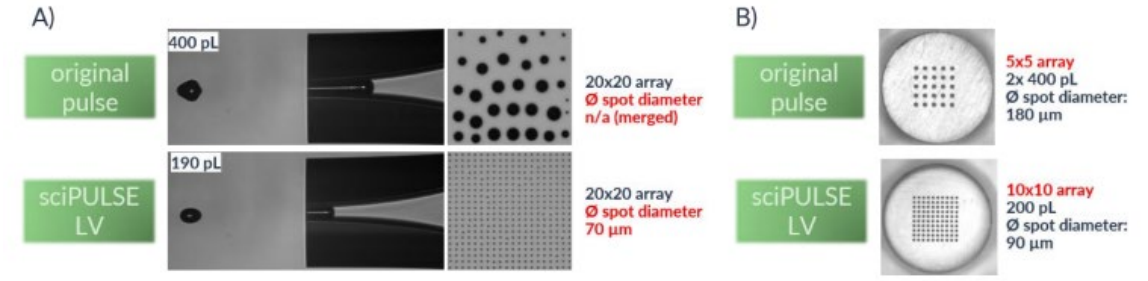

Figure 3. Comparison of printing with original standard pulse and sciPULSE LOW VOLUME. A) Printed array (20x20) with a spot pitch of 150 µm on a hydrophobic glass slide. Due to the narrow spot pitch the spots merge with original setup whereas result in a uniform array with sciPULSE LOW VOLUME. B) Printing in a Gentix PS 384 well plate with an array of 5x5 with a dot pitch of 300 µm standard settings and an array of 10x10 with a dot pitch of 160 µm with sciPULSE LV.

In another approach (Figure 3B), the numbers of spots that can be printed on a

defined area were compared by using either the standard pulse or the sciPULSE LOW VOLUME settings. In a 384 PS well plate (Genetix), 5x5 arrays with a spot pitch of 300 Âµm were printed into one well (standard pulse shape). The spot diameters were found to be 180 Âµm (Figure 3B top panel). Applying sciPULSE LOW VOLUME allows the array to be much narrower – on the same area, arrays of 10x10 spots with a spot pitch of 160 µm were printed (Figure 3B, bottom panel). Thus, sciPULSE LOW VOLUME enables the possibility to increase the number of reaction sites or replicates in the well plate to extend multiplexing capacities.

Conclusion and future direction

- sciPULSE is an easy plug-in device to go beyond the standard parameters such as droplet volumes, spot diameter or viscosity of the currently available medium sized PDCs.
- Using sciPULSE LOW VOLUME enables the reduction of droplet volumes to 150 -220 pL with SCIENION's PDC 80
- Using the sciPULSE LOW VOLUME enables the reduction of spot diameters down to 70 µm
- Smaller drop volumes and spot diameter allow for the increased number of spots on a certain area with regards to multiplex applications
- sciPULSE LOW VOLUME is a standardized application package that reduces the required validation times of protocols and thus significantly reduces the overall costs for the implementation

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