

sciFLEXARRAYER Application Note No. 08010

Performance evaluation for SCIENIONs sciDROP PICO and sciDROP NANO technology using the Artel dual-dye ratiometric photometry

An essential component of today's research in life science is the use of automated liquid handling equipment. The transferred volumes are becoming increasingly small down to nano- and picoliter range. Verifying the absolute transferred volume and the well-to-well reproducibility is important to ensure the quality of the process but there are only a few methods available that can quickly and accurately measure dispensed volumes in this low range. The transfer performance of the sciFLEXARRAYER equipped with a) the sciDROP PICO and b) the sciDROP NANO technology was assessed with the Artel MVS® Multichannel Verification System, a standardized and traceable volume verification platform using a dual-dye absorbance based method. The sciDROP PICO is a piezo based non-contact dispenser for ultra-low volume liquid handling (pico to nanoliter). The sciDROP NANO is a solenoid valve based dispensing technology that is capable of dispensing drops in the range of 50 – 200 nl per drop. Both technologies employ an internal imaging system for volume measurement.

Two assay specific reagents, aqueous and DMSO have been implemented in this study representing how the user may assess volume accuracy with aqueous solutions and organic solvents. Dispensed volumes of aqueous-based dye solutions between 20 and 1000 nl (600 nl) were analyzed. Data showed a very good correlation of measured accuracies between both systems. Precision data revealed a very high reproducibility of the piezo dispenser with a CV below 2%. It is also demonstrated that volume verification can be performed by using assay specific reagents such as dimethyl sulfoxide (DMSO). The results presented here show outstanding accuracy and reproducibility of the sciFLEXARRAYER in dispensing of volumes ranging from 5 nl to 1000 nl.

Materials and methods

SCIENIONs two liquid handling technologies the sciDROP PICO and sciDROP NANO cover a dispensing range from picoliter to microliter. The sciDROP PICO system uses Piezo Dispense Capillaries (PDC) and is mainly used in aspirate/dispense applications in the ultra-low volume range (100 picoliter up to a few nanoliter). The sciDROP NANO system is mainly used in bulk dispensing of liquids from the nanoliter to microliter range. Both technologies can be used for water based samples as well as DMSO or organic based samples. In sciFLEXARRAYERs, the volume of a droplet is calculated by an efficient algorithm: the sciDROPVOLUME. The Software enables, as a further option, a precise drop adaption to the requested drop volume by using use an "AutoTuning" module. The measurement is established in real time as it passes the standard drop-detection camera (Figure 1). With standard settings, the systematic error of sciDROPVOLUME is less than 2.0%. By calibrating specific to the used assay (e.g. by using the MVS technology) the systematic error can be further minimized. The typical systematic error in a well-tuned system is 10-fold less.

In this publication the visual drop volume technology of the sciFLEXARRAYER was evaluated by the well-established MVS method from Artel.

The dual-dye ratiometric photometry method employed by Artel's MVS® utilizes two dyes with distinct absorbance maxima at 520 nm (red) and 730 nm (blue). First, MVS sample solutions containing different concentrations of the red dye but identical concentrations of the blue dye are dispensed into 96- or 384-well Artel verification plates over a volume range of 10 nl to 350 µl. Subsequently, MVS diluent solution containing a fixed concentration of the blue dye that equals its concentration in the sample solution is added to fill the wells. Consequently, red dye concentrations vary whereas blue dye concentrations are equal, serving as an internal standard for calculating absorbance path lengths with the Beer-Lambert Law. Finally, these calculated path lengths were used to precisely determine concentrations of the red dye and hence precision and accuracy of pipetted sample volumes (Figure 2).

MVS uses routinely aqueous based sample solutions. Biochemical and pharmaceutical research sometimes also requires use of non-aqueous solutions such as DMSO. To analyze the performance of the sciDROP PICO and the sciDROP NANO technologies two types of MVS sample solutions, aqueous and 100% DMSO, were used.



Fig.1 Autodrop detection with calculated drop volume (drop volume 350pl)

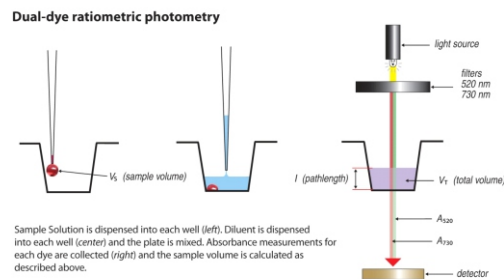


Fig.2 Principle of dual dye photometry

Results and discussion

Aqueous solution with sciDROP PICO

In the volume-on-demand mode a target volume was programmed and the SCIENION Software measured the drop volume of the respective PDC and automatically calculated the number of drops needed to meet the target volume. Two PDC were used in parallel channels to show an inter-channel reproducibility and accuracy of the sciDROP PICO technology. Both PDCs exhibit very good accuracy and reproducibility. In the specified measurement range of the MVS the CV is below 2.0 % for both PDCs as shown in Figure 3a and b.

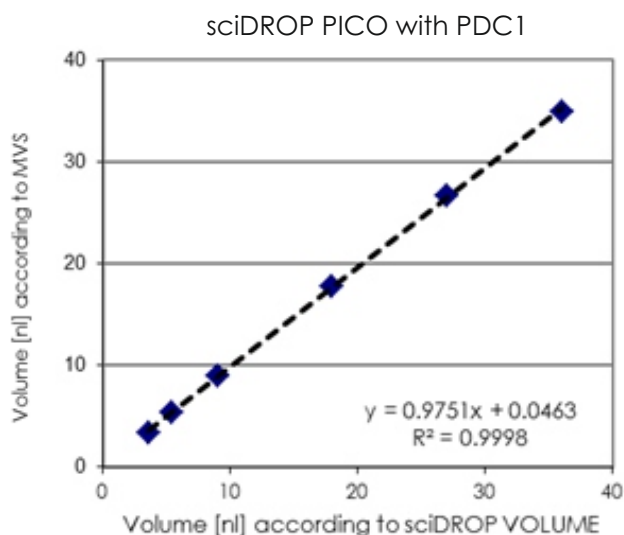


Fig.3a PDC1: sciDROP PICO water based solution in a aspirate/dispense mode; MVS drop volume vs. sciDROP VOLUME

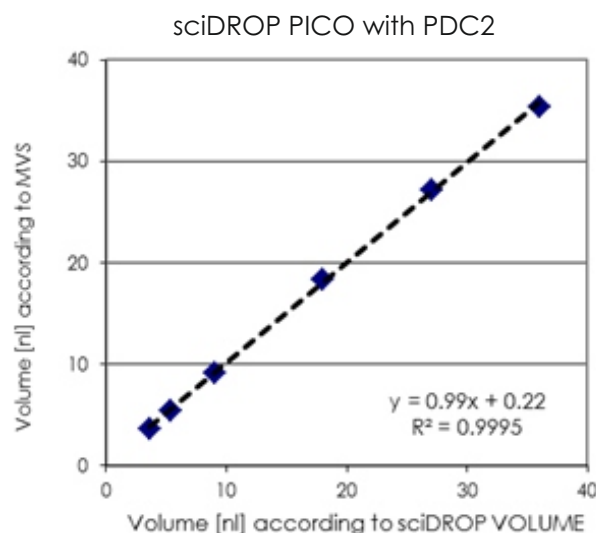


Fig.3b PDC2: sciDROP PICO water based solution in a aspirate/dispense mode; MVS drop volume vs. sciDROP VOLUME

Aqueous solution with sciDROP NANO

The machine was equipped for the aspirate / dispense mode. Prior to dispensing, the drop volume was measured resulting in 89.4 nl. This was taken as basis to calculate the required number of drops for each well. The graph shows a linearity with a slight positive offset around 2 % for all numbers of drops dispensed (Figure 4a). A mean of 0.82 % CV showed a very good stability of drop volume during dispensing.

The sciDROP NANO technology is usually used in the bulk dispensing mode. For using the sciNANO in the bulk dispensing mode another setup was used with a different drop volume of 32 nl per drop instead. The graph showed good linearity (Figure 4b) and with a mean of 1.13 % the CV also illustrated very good stability of drop volume during dispensing. The results for the sciDROP NANO technology are shown in figure 4a+b.

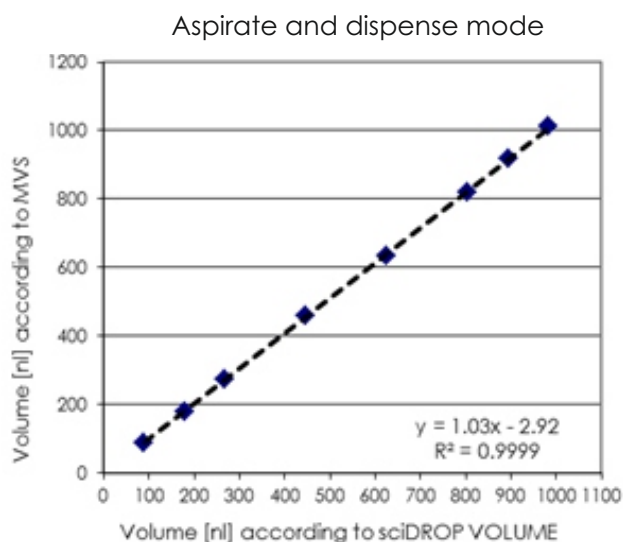


Fig.4a sciDROP NANO water based solution, aspirate / dispense

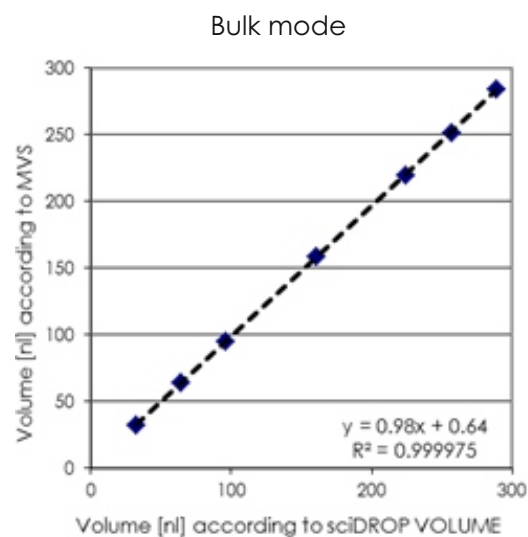


Fig.4b sciDROP NANO water based solution, bulk dispensing

DMSO solutions

Also with Artel DMSO sample solutions the SCIENION system showed very good reproducibility and accuracy (Figure 5a+b). Mean CV for all target volumes was 1.6 %.

Before dispensing, the drop volume was measured resulting in 32.1 nl. This was taken as basis to calculate the required number of drops for each well. The graph showed also perfect linearity for all numbers of drops dispensed (Figure 5b) and with a mean of 1.24 % CV showed very good stability of drop volume during dispensing.

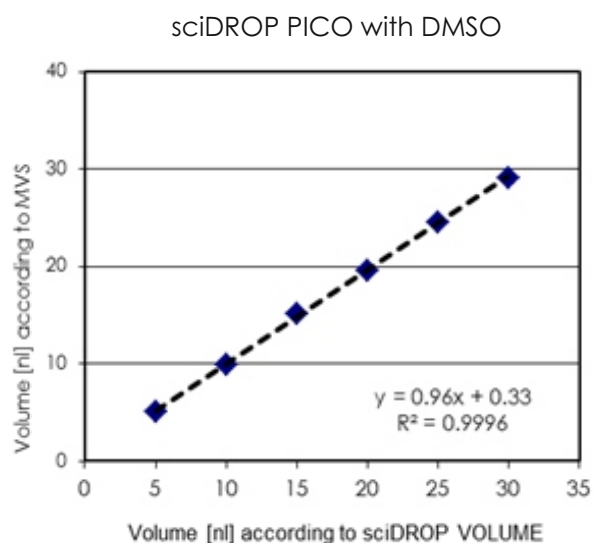


Fig.5a sciDROP PICO DMSO based solution, aspirate / dispense

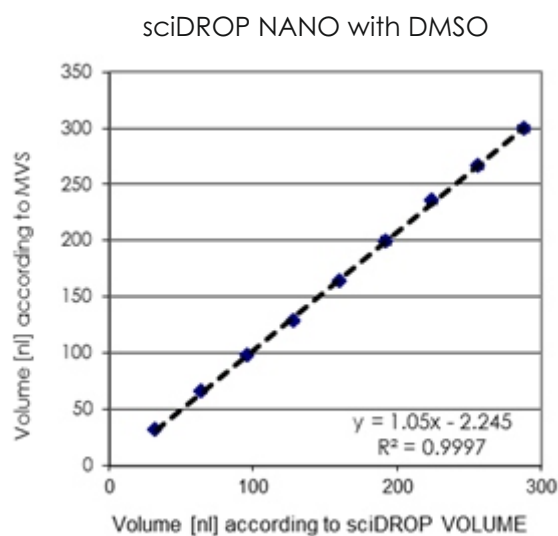


Fig.5b sciDROP NANO DMSO based solution, bulk dispensing mode dispense

sciDROP technology offer a wide volume range

This can be visualized when combining the results of sciDROP Pico and sciDROP NANO Systems. When using DMSO based samples, precision and accuracy are excellent for both technologies over the full volume range from picoliter to nanoliter. With the sciDROP PICO the optical measurement ensures a precision of CV < 2%. Due to the bigger drop shape/size in the Nano, a different drop camera resolution needed to be utilized. This resulted in a slightly higher CV value when comparing the Pico with the Nano (CV < 5%) (Figure 6)

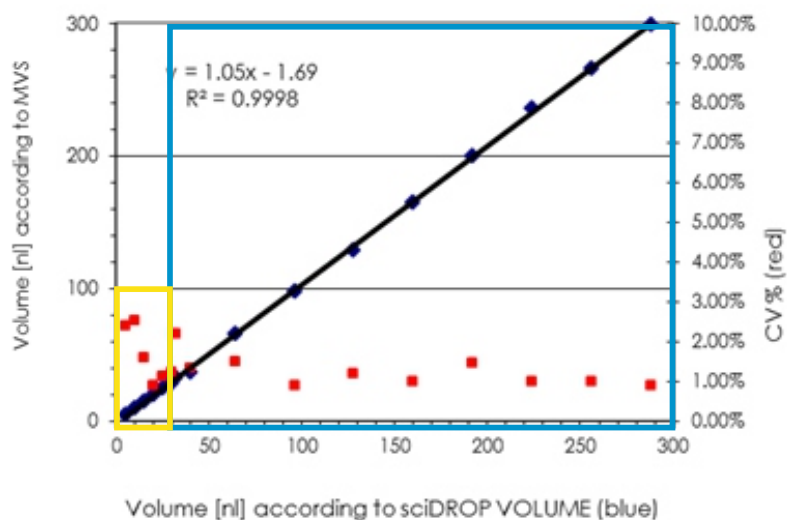


Fig.6 sciDROP PICO (yellow) and sciDROP NANO (blue) DMSO based solution, combined results

Conclusion

The data presented show a high level of correlation between the actually dispensed and the calculated volumes for the measured range when dispensing aqueous sample solutions (e.g. biological samples: DNA, RNA, proteins) as well as DMSO (e.g. compounds, peptides). This demonstrates consistent performance of the SCIENION technologies sciDROP PICO and sciDROP NANO. It could be shown that 5 nl is already enough for linear and sensitive measurements for sciDROP system as well as the MVS system of ARTEL.

The combined SCIENION technologies ensure linearity in dispensing and very high reproducibility over the full range of 5 nl to 290 nl in the ultra-low volume range from pico-to nanoliter (5-30 nl sciDROP PICO, 32-290 nl sciDROP NANO) with CVs below 2% for sciDROP PICO and below 5% for the sciDROP NANO system as shown in figure 6. Artel ratiometric photometry is a reliable and easily applicable method to analyze volume dispenses even in the low nL range.