Assessing Nanoliter Transfer Performance for Scienion Ultra-Low-Volume Liquid Handler Using the Artel dual-dye ratiometric photometry

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Abstract

An essential component of today's research in life science is the use of automated liquid handling equipment. Along with an increase in use of automated liquid handlers, transferred volumes have become increasingly small. Volume transfers in the nano- and picoliter range are meanwhile done routinely. Verifying the absolute transferred volume and the well-to-well reproducibility is important to ensure the quality of the process, but there are few methods available that can quickly and accurately interrogate dispensed volumes in this range.

In this presentation, the transfer performance of the sciFLEXARRAYER® (Scienion AG) was assessed with the Artel (MVS®) Multichannel Verification System, a standardized and traceable volume verification platform using a dual-dye absorbance based method. The sciFLEXARRAYER® is a piezo based non-contact dispensing system for ultra-low volume liquid handling. It comprises an internal imaging system for volume measurement.

Dispensed volumes of aqueous-based dye solutions between 20 and 1000 nl (600 nl) were analysed. Data show a very good correlation of measured accuracy between both systems. Precision data revealed a very high reproducibility of the piezo dispenser with a CV below 4% (2%). It is also demonstrated that volume verification can be performed by using assay specific reagents such as dimethyl sulfoxide.

Introduction

A volume verification method, which can be used to quantify the amount of transferred volume from a liquid handler, is an essential component that enables proper interpretation of experimental results (reference 1).
If the volume verification procedures are scientifically-based and the methods are properly executed, then the verification method can be used to increase confidence in assay integrity and liquid handler performance. A very important facet of understanding liquid handler performance is by properly executing both the liquid handler task and the volume verification method. If care is not taken when performing the liquid handler dispense protocol, or the volume verification method is not properly implemented, true liquid handler performance may not be measured.

The sciFLEXARRAYERs are designed to dispense droplets in non-contact manner. The dispense is driven by an piezo actuator. For best performance, the sample solution must be free of air bubbles and dust. For photometric verification methods, such as with MVS (reference 2), best performance also requires bubble free, properly mixed solutions which are in thermal equilibrium with the lab environment. In addition, proper sample mixing is important and the key to accurate dispensing performance confirmation for a liquid handler by means of MVS. This presentation demonstrates sciFLEXARRAYER volume transfer performance with the MVS when both methods are properly implemented and executed.

Experimental Section

MVS dual dye photometry

The dual-dye ratiometric photometry method employed by the MVS utilizes two dyes with distinct absorbance maxima at 520nm (red) and 730nm (blue). Six aqueous based sample solutions containing different concentrations of the red dye are used for testing the performance of instruments under standard conditions dispensing into 96- and 384-well plates over and entire volume range of 10 nL to 350 µL. The concentration of blue dye is constant in all sample solutions across the volume ranges and is equal to that of a diluent buffer. The blue dye is therefore used as an internal standard to calculate solution depth in each well. The liquid handler to be tested is used to dispense sample solution (and diluent if appropriate) into the wells of a microtiter plate, and the absorbance at both wavelengths is measured for every well. By applying the Beer-Lambert law, the MVS uses absorbance values and automatically calculates both the precision and accuracy of the volume delivered by each pipetting channel.

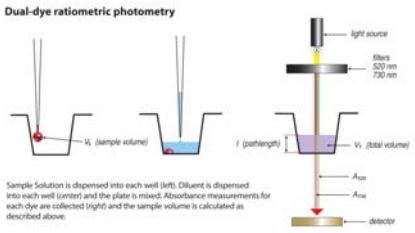

Figure 1. Principle of dual dye photometry

Biochemical and pharmaceutical research sometimes also requires use of non aqueous solutions or solutions containing a considerable amount of organic solvents such as DMSO. For these applications a MVS red dye stock solution is gravimetrically combined with the solvent (e.g. DMSO) according to Artel's solution helper software. This approach enables to test liquid handlers by using buffer components / solvents comparable to conditions of a real assay. Both type of solutions, aqueous and solvent (DMSO) based, were applied to analyze sciLEXARRAYER performance.

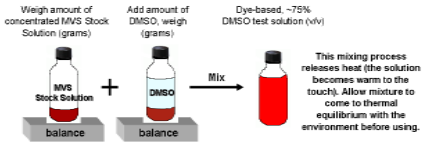

Figure 2. Overview of simple preparation of solvent (DMSO) based test solution for MVS volume verification

sciFLEXARRAYER

- In sciFLEXARRAYERs, volume of a droplet is calculated by an efficient algorithm: the sciDROPVOLUME. The measurement is established in real time as it passes the standard drop-detection camera.
- With standard settings, the systematic error of sciDROPVOLUME is less than 3,5%. By calibrating (e.g. by using the MVS technology) the systematic error can be minimized **Figure 3**.
- All sciFLEXARRAYERS offer non-contact printing with high accuracy and precision.
- The systems can be used for various applications: DNA, Proteins, Glycans, MALDI targets, Biosensors, etc.
- The sciFLEX technology allows for ELISA MULTIPLEXING by enabling dispensing of up to 144 samples in a single well of a 96-well MTP **Figure 4**. Other SBS format MTPs can also be addressed up to 9600-well plates.
- **Ultra-low dispensing volume with adjustable drop volume:** The heart of the system, a glass piezo dispensing capillary (PDC), can dispense drops with various volumes. The volume ranges from as low as 50 picoliter up to 800 picoliter per drop. In combination with the high dispense frequency used by the system, volumes of up to 1 microliter (µl) per second can be achieved **Figure 5**.
- **Walk-away systems:** All systems include rapid microstructure scanning for target fiducial detection and following dispensing with highly accurate positioning of substances. The high sensitivity of the camera enables detection of dried spots giving the ability of a complete Quality control of the spotted target **Figure 6**.
- **One technology from R&D to production:** The product line comprises small machines for R&D purposes (sciFLEXARRAYER DW) and reaches to high-throughput in-line production machines (sciFLEXARRAYER S100) **Figure 7.** The user-friendly integration of dedicated hard- and software tools offers reproducibly high production quality and reliable in-process quality control.

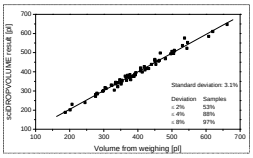

Figure 3. The graph shows the high linear correlation between the results from the software module and the weighted volumes in a broad range of available droplet sizes (CV=3.1%).

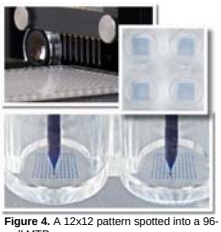

Figure 4. A 12x12 pattern spotted into a 96-well MTP.

Figure 5. The same PDC dispensing a wide range of volumes (350, 500, 780 pl)

Figure 7. The S100. the ultimative high-throughput in-line production machine.

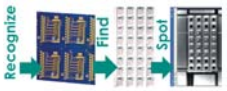

Figure 6. Automatic fiducial detection with subsequent spotting and quality control.

About Scienion AG: provides systems and services for the contact-free printing of biological and chemical agents for diagnostics, pharmaceutics, veterinary, plant and food analytics and research. Systems and software are characterized by their versatility, precision and robustness. The company is a renowned specialist for ultra low volume liquid handling, particularly for the handling of precious and sensitive compounds of biological or chemical origin.

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Results and Summary

Accuracy and Precision results using aqueous solutions

MVS was used to rapidly assess sciFLEXARRAYER for various target volumes in the range from 20nl to 1000nl with MVS aqueous test solutions. The performance of the sciFLEXARRAYER was tested for linearity over the entire volume range. In addition the results of the MVS volumes determination were compared with the theroetical volumes based on the analysis of drop size and corresponding number of drops for each dispensed volume. **Figure 8** demonstrates a significantly linear volume delivery, as measured by the MVS for each of the 24 replicate volumes. **Figure 9** shows a good agreement of the determined volume when both volume verification methods are compared.

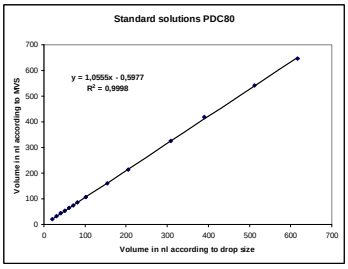


Figure 8. MVS measured volumes vs. theoretical target volumes delivered by the sciFLEXARRAYER show a linear relationship over the tested volume range for the aqueous test solutions.

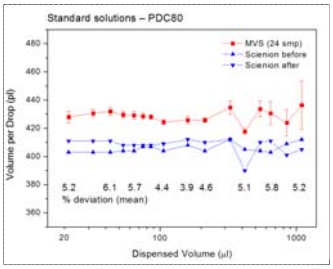


Figure 9. Comparison of the average volumes show a good correlation between the two methods. Relative inaccuracy compared to MVS is nearly constant around 5% over the entire volume range indicating a systematic difference between the two methods. Please note that in the low volume range (20 -200 nl) CVs are below 1% and rise only moderately to 2% at 600nl and around 4 % at 1000 nl demonstrating the high repeatability of the piezo dispenser.

Accuracy and Precision results using DMSO

Same assessment as described for aqueous solutions was performed with DMSO test solution.

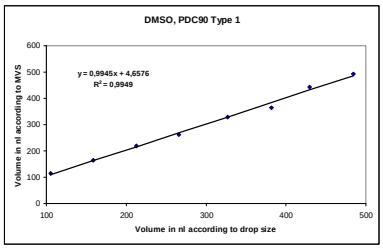


Figure 10. MVS measured volumes vs. theoretical target volumes delivered by the sciFLEXARRAYER show also a linear relationship over the tested volume range for the DMSO test solutions.

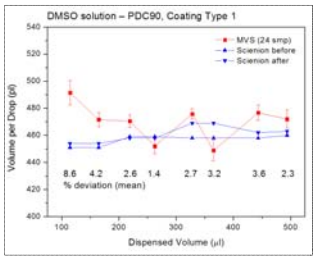
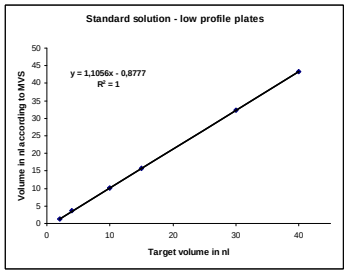


Figure 11. Comparison of the average volumes of DMSO test solutions reveal a good correlation and demonstrate feasibility of the piezo dispenser to use non-aqueous solvents.

Monitoring applicability of ratiometric photometry in the ultra low volume range

MVS delivers NIST traceable results in the volume range of 30nl – 350µl using MVS verification plates. It is also suitable to monitor volumes down to 10nl using low profile plates (Greiner). Since sciFLEXARRAYER is especially suited for ultra low volume delivery, volume verification with MVS was tested in this low range to demonstrate reliability of ratiometric photometry even below specification limits.



Target volume nl	2	4	10	15	30	40
CV % (n=24)	8.33	2.78	1.96	1.27	0.93	0.92

Figure 12. MVS measured volumes vs. theoretical target volumes delivered by the sciFLEXARRAYER show a linear relationship even below the specification limits of MVS. CV demonstrate excellent repeatability of sciFLEXARRAYER, the increase at 2nl displays the detection limit of the MVS. (Yellow: below MVS specifications)

References

(1) Albert and Bradshaw, *J. Assoc. Lab. Autom.*, **2007**, *12*, 172-180
(2) Bradshaw *et al. J. Assoc. Lab. Autom.*, **2005**, *10*, 35-42

Conclusion

Data collected shows a high level of correlation between the actual volumes dispensed and the calculated volumes for the measured range when dispensing aqueous sample solutions and DMSO thereby demonstrating consistent performance of the sciFLEXARRAYER with these solvents. Capillary piezo dispensing ensures very high reproducibility in the ultra low volume range. Ratiometric photometry is a reliable and quick method to analyze volume dispenses even in the low nl range.

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