In collaboration with

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Enhanced Calibration of CERTUS FLEX Dispenser Using LUMINA for Accurate and Efficient Liquid Handling

Introduction

Dispensers and pipetting devices require regular calibration to ensure consistent liquid handling performance. Factors such as fluid properties, environmental conditions (e.g., laboratory temperature), and device wear can significantly impact dispensing accuracy. Traditional calibration methods, including gravimetric weighing and dye-based absorbance, present several challenges:

Gravimetric Calibration: Requires repetitive dispensing to achieve measurable weight differences on high-precision scales. This process is time-intensive, assumes homogeneity across dispense cycles, and is impractical for small volumes.

Dye-Based Calibration: While capable of measuring smaller volumes, this method necessitates the preparation of standard curves and uses surrogate liquids, which may not reflect experimental conditions.

These methods are labor-intensive and less suited for high-throughput workflows. The LUMINA device overcomes these limitations by directly measuring the dispensed volumes of the intended liquid (e.g., DMSO, Cell Media or PCR reagents), allowing for faster calibration, real-time adjustments, and superior accuracy.

Materials and Methods

Equipment

- CERTUS FLEX Dispenser (nNano AG)
- LUMINA Volume Measurement System (Liquimetrix)
- 1536-well plate

Dispensing Setup

- 4 Gyger dispensing valves: CH5 (ø0.20/0.10), H6 (ø0.10/0.03), CH7 (ø0.15/0.06), CH8 (ø0.15/0.06)
- Target volumes: 2–10 µL
- Liquid: DMSO

Calibration Process

Factory Calibration: Factory calibration for water was used intentionally with DMSO to create systematic deviations in dispensing accuracy and precision. This setup highlights the necessity and effectiveness of recalibration.

LUMINA Calibration: LUMINA directly measured the dispensed DMSO volumes in the 1536-well plate, providing real-time feedback for each valve and enabling precise adjustments. For each target volume, the mean of 16 measurements was used for calibration. These 16 volumes were distributed across the plate in a predefined pattern to minimize the influence of plate-specific or evaporation effects on the calibration process.

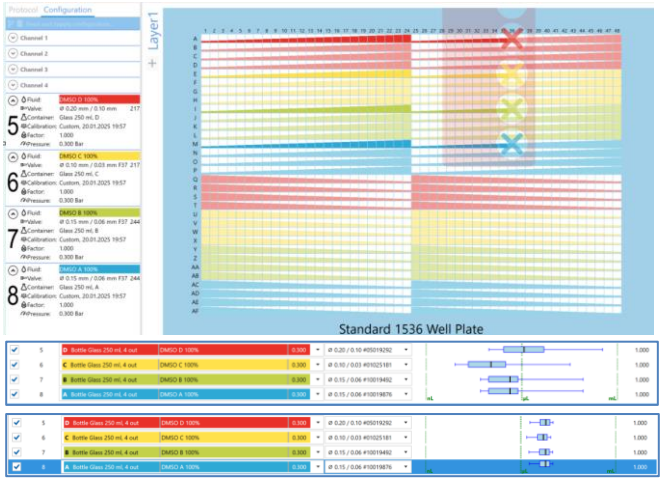


Figure 1 Dispensing Pattern and calibration range with factory calibration (water) and after LUMINA calibration.

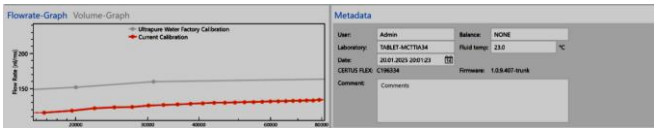


Figure 2 Calibration Curve with 24 support points

After calibration with LUMINA, the calibrated volume range is narrower and specifically tailored to the working volume of the plate. However, this range is calibrated with 24 support points, compared to only 2 for the same volume range in the factory calibration, resulting in significantly improved precision and accuracy.

Results and Discussion

Factory Calibration Results

Factory calibration using water produced significant volume deviations when dispensing DMSO, with systematic under-dispensing observed across all channels (CH5–CH8).

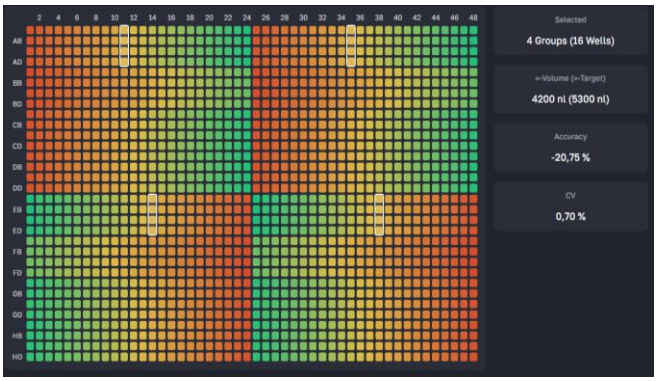


Figure 3 LUMINA SW showing CV & ACC for CH5 at 5,3µl

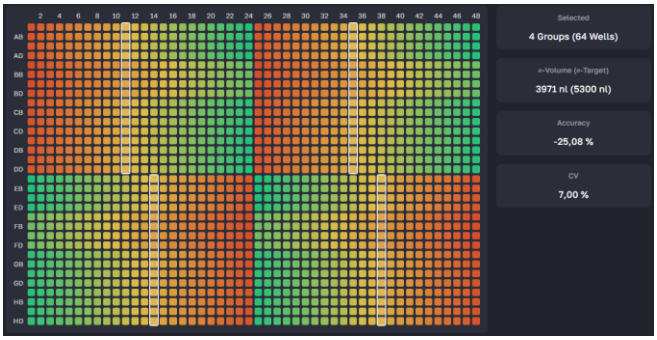
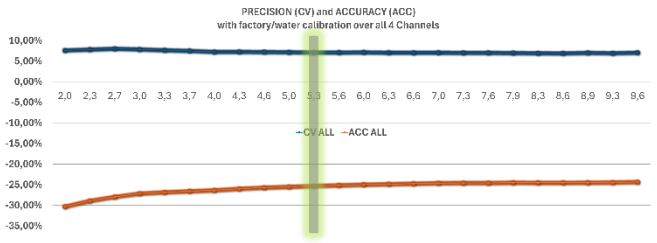


Figure 4 CV & ACC over all Channels at 5,3µl



Graph 1 CV & ACC over all Channels & Volumes

CV values were elevated, particularly for lower volumes, where deviations in individual wells were more pronounced. Accuracy errors exceeded 20%, demonstrating the incompatibility of water-based calibration when working with different liquids like DMSO.

LUMINA Calibration Results

LUMINA calibration reduced accuracy errors to within ±1% across all target volumes, even at lower volumes where measurement precision is more challenging.

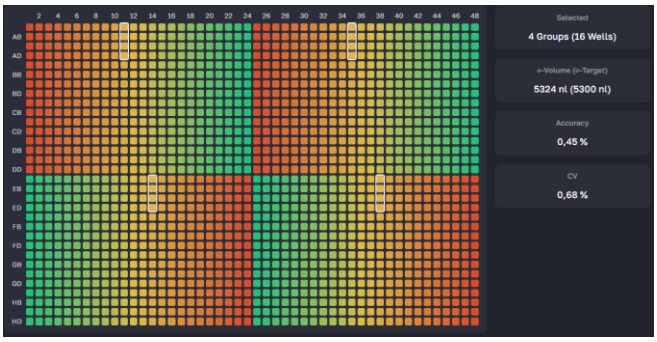


Figure 5 CV & ACC for CH5 at 5,3µl after Calibration with LUMINA

CV values improved significantly, with most values below 1%, demonstrating the high consistency achieved for all channels.

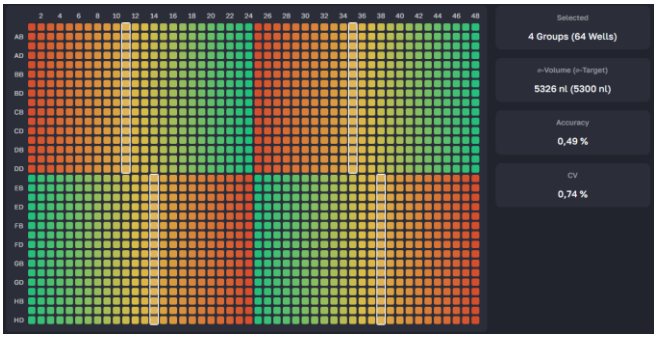
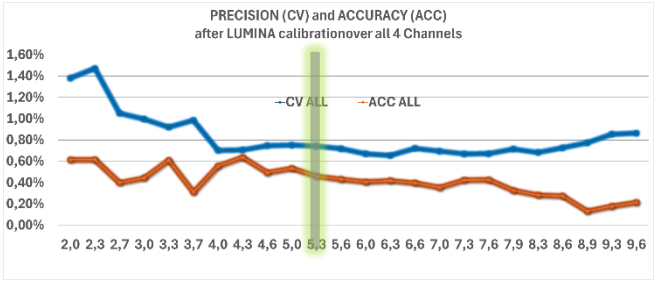


Figure 6 CV & ACC over all Channels at 5,3µl after Calibration with LUMINA



Graph 2 Graph 1 CV & ACC over all Channels & Volumes after Calibration with LUMINA

The spatial distribution of the 16 measurement points across the plate minimized potential effects from evaporation or plate-specific variations, further contributing to the robustness of the calibration.

Following LUMINA calibration, not only is each channel individually optimized to achieve perfect accuracy, but all four channels are also precisely aligned with one another, ensuring consistent and harmonized dispensing behavior across the entire system.

Conclusion

These valves were calibrated in a single step to achieve consistent performance across all channels, despite their differing configurations. This demonstrates the capability of LUMINA to harmonize dispensing behavior efficiently and accurately across a variety of fluidic setups. The same valves and calibration approach were applied in Application Note #2501, where calibrated channels were successfully used to perform backfills to compensate for evaporation effects. This underscores the versatility of the LUMINA system in addressing diverse liquid handling challenges

Time Savings:

Traditional calibration methods require multiple weighing steps for each calibration point. For example, calibrating a single valve for a single volume typically involves the following:

1. Dispense the volume (e.g., 100 × 1 µL) into a tared vial.
2. Weigh the vial to determine the total dispensed volume.
3. Calculate the per-dispense volume.
4. Adjust the dispenser's settings and repeat if the result is not satisfactory.

Each calibration point (or "support point") for a single valve typically takes 5–10 minutes. To calibrate a single channel over a broader volume range under typical conditions, this process usually takes 20–60 minutes, depending on the number of support points and the precision required. For a complex setup involving multiple channels and a high number of support points, this time can increase significantly.

For example, calibrating 4 valves, each with 24 support points, would traditionally require approximately 2–4 hours of manual work. This includes vial preparation, weighing, data entry, and iterative adjustments.

In contrast, the LUMINA system allows for simultaneous calibration of all support points across multiple channels. In this study, 4 channels were calibrated with 24 support points each in a single measurement, completing the entire process in just **under 8 minutes**. This represents a **time savings of over 90%** compared to traditional methods.

This dramatic efficiency gain not only reduces labor but also minimizes downtime for critical equipment, making LUMINA an ideal solution for high-throughput laboratories. By directly measuring the dispensed volume of DMSO and averaging measurements distributed across the plate, LUMINA ensures that calibration results align with actual experimental conditions. In addition to achieving superior accuracy and consistency, LUMINA significantly streamlines the calibration process, supporting robust and efficient high-throughput applications.

Outlook

The results of this study underscore the potential of LUMINA not only for standalone calibration tasks but also as an integral component in automated laboratory workflows. LUMINA's ability to perform both dispenser verification and calibration inline or online can be seamlessly integrated into automated processes. This eliminates the need for manual intervention, significantly reducing time and labor while ensuring consistently high accuracy and precision. In pharmaceutical and diagnostic laboratory automation, such capabilities can profoundly impact quality assurance and workflow efficiency. Automated inline calibration ensures that dispensers consistently operate within defined tolerances, reducing variability in assay results. Online process control allows for real-time adjustments during experiments, minimizing waste of costly reagents and consumables.

By incorporating LUMINA into fully automated systems, laboratories can unlock a new level of reliability and throughput, paving the way for more efficient and scalable processes in pharmaceutical development, diagnostics, and beyond.

Acknowledgements

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