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Contactless Volume Verification in Microwell Plates: Precision Monitoring Across Liquid Types and Plate Formats

Background

Almost every single biological assay in microtiter plates and microarrays is volume-dependent, thus the smallest deviations in volume can have a huge impact on an experiment's result. With an increasing demand for process monitoring and quality assurance in today's laboratory automation, the need for volume verification systems becomes greater than ever. At the same time, the trend towards assay miniaturization decreases the volume per sample, while the overall number of samples increases rapidly. Existing technologies for volume verification do not meet these growing requirements or can simple not be adapted towards modern sample carriers, such as 1536 well plates or microarrays.

It is generally known that the influence of evaporation or absorption of water from the air cannot be neglected, especially with small volumes presented in HTS microtiter plates. LUMINA allows the accurate measurement of volume in each individual well of microwell plates with up to 1536 wells. The process time of less than a minute per plate is very fast, allowing the demonstration of evaporation or absorption kinematics over time.

In this application note, we present an experiment, which not only allows the assessment of relative measurement accuracy, but also demonstrates the impact of evaporation and absorption on virtually any assay.

Methods

All experiments were performed with two microwell plates (Greiner 1536 Lumitrac HiBase Medium Binding, Ref. No. 782075), one plate filled with distilled water and one plate filled with 100% DMSO. Each well of a column is filled with the same volume. Starting at column 1 with a volume of 2000nl, the filling volume is increased by 268nl in each column, up to column 24 where a target volume of 8164nl is dispensed. Columns 25 - 48 are filled in the same pattern starting with 2000nl up to 8164nl (see Figure 1). For dispensation of the liquids, SPT Labtech dragonfly® discovery was used.

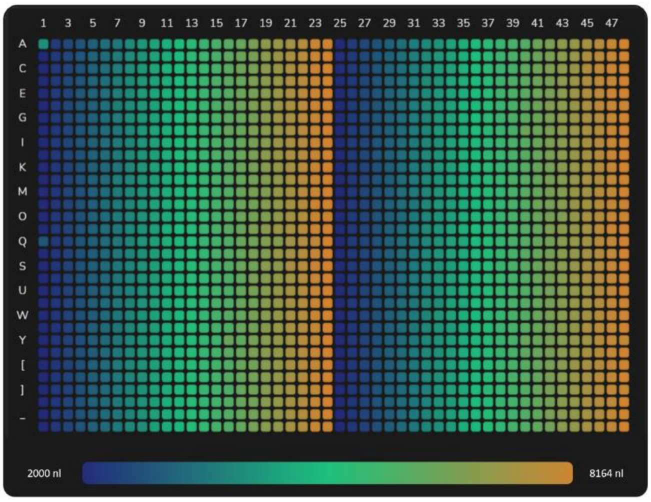

Figure 1: Dispensing pattern for the target plates

After dispensation, each plate is centrifuged for one minute at 300rcf. Subsequently, six measurements (M1 to M6) for each plate are done with the volume measurement system LUMINA. The period between two consecutive measurements of one plate is approx. 200 s to 500 s.

After the third measurement (M3) of each plate, stainless steel precision spheres with an exact volume of 268nl are added to four wells (H16, H40, Y16, Y40). Then the remaining three measurements (M4-M6) are carried out. The addition of the precision spheres adds a very deterministic and invariant volume to the wells, which can be evaluated by subtracting the absolute volumes after and before the spheres where added. This allows an

Figure 2: Design of Experiment

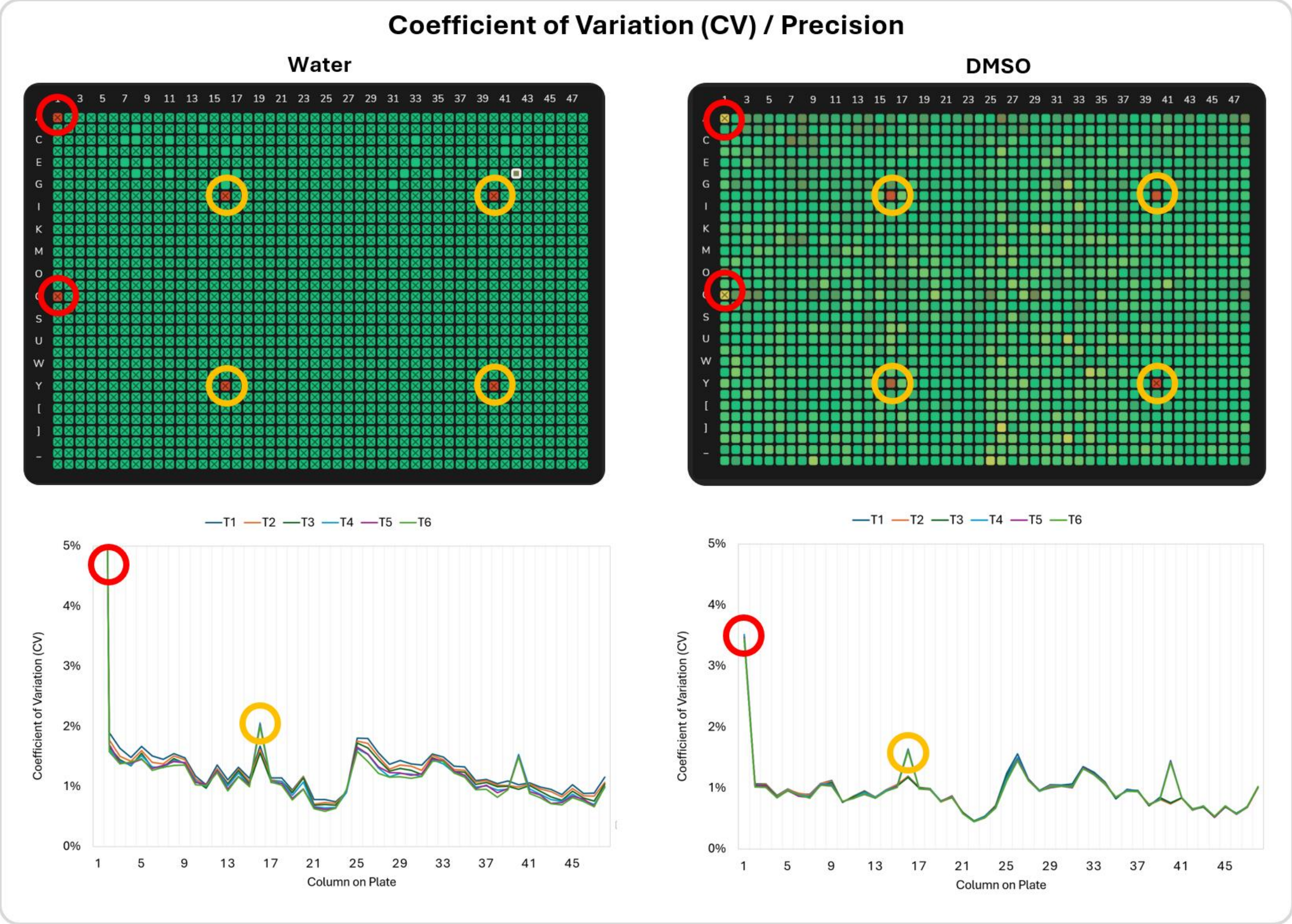

Figure 3: Coefficient of Variation (CV) for Water (left) and DMSO (right) across each column of the plate (bottom left and right). The red circles indicate dispensing errors which impact CV significantly, the yellow circles highlight the wells with added precision spheres, which distort the measured CV as well.

accurate estimated of the relative measurement accuracy. The described process can be obtained from Figure 2.

Table 1 shows the exact timestamp of measurement in the LUMINA system for each type of plate. The timestamp T0 represents the time of dispensation.

Table 1: Timestamps for the measurements

	Timestamp (seconds)	
Step	Water	DMSO
Dispensing (T0)	0	0
M1	570	600
M2	870	780
M3	1050	1020
M4	1350	1440
M5	1530	4620
M6	1710	1800

Results

In the following, we will discuss the measurement results of the LUMINA system in terms of precision and accuracy

Coefficient of Variation (CV)

The coefficient of variation (CV) represents the dispensing precision, and is typically calculated as follows

$$\mathrm{CV} = \frac{Standard\ Deviation}{Mean}$$

The CV describes how closely the measurement values are clustered together, without providing any information about the absolute accuracy of the dispensation.

The results for the CVs, calculated for each row of the plate can be obtained from Figure 3.

For both, water and DMSO, the CV ranges between 1 % and 2 %, whereas higher volumes lead to a lower CV. The significantly higher volumes in A1 and Q1 are due to a usage error of the dispenser, caused by a hanging droplet at the dispensing tip which fell into these wells upon process start. Furthermore, columns 16 and 40 also show an increased CV for the measurements M4 to M6, which is due to the fact that the precision spheres where added to some of the wells in these columns.

Accuracy

The accuracy describes, how close the measurement values are to the expected value, and is typically expressed as:

$$\text{Accuracy} = \frac{Mean - Target}{Target}$$

Figure 5 shows the accuracy results from the six measurements. For both liquids, water and DMSO, the dispensing accuracy increases with higher volumes.

The effect of evaporation of water can be easily observed from the left graph, as the accuracy decreases over time. This effect is stronger for columns with lower volumes, which leads to the conclusion that the magnitude of evaporation is volume-dependent.

Furthermore, we can interpolate the theoretical volume for the timestamp T0, since we do know the time delay between the individual measurements as well as the measurement volume in the columns. The accuracy for the timestamp T0, which represents the time of dispensation, the accuracy ranges between +1 % and -1%, for both, water and DMSO.

Based on its physical properties, DMSO does not evaporate, but rather absorbs water from the

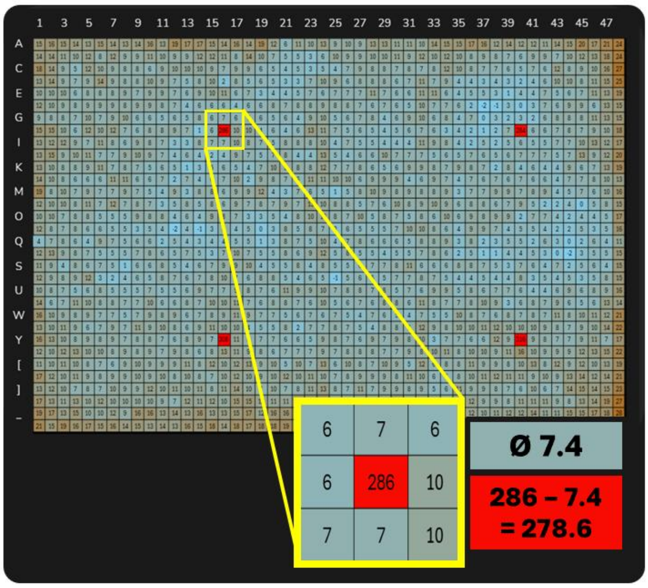

Figure 4: Relative volume increase (DMSO) between two consecutive measurements (M3 & M4) for DMSO.

surrounding air, which leads to an increase of volume over time. This effect is not as strong as evaporation, however it can also be observed in the right graph.

Both evaporation and absorption highly depend on the environmental conditions of the experiment, such as temperature, humidity and the amount of air-flow above the assay plate. However the absolute volume within the wells, as well as the presence of a lid both highly impact the volume kinematics.

Relative Measurement accuracy

In order to demonstrate the relative measurement accuracy of the LUMINA system, we added precision spheres with an absolute volume of 268 nl to four wells of each plate after the third measurement (M3).

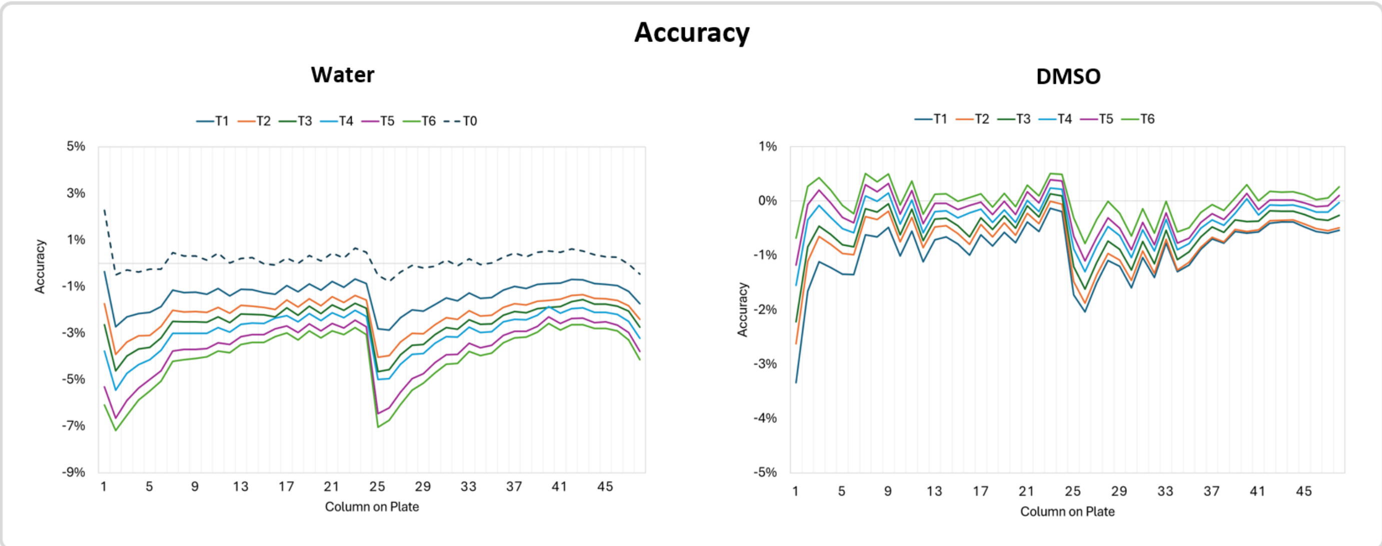

Figure 5: Accuracy for Water (left) and DMSO (right) across each column of the plate for different timestamps (T1 – T6). The left graph additionally shows the interpolated measurement accuracy for T0.

Figure 4 shows the absolute volume difference between each well of the plate between measurement 4 and measurement 3. The average volume increase is approx. 10nl across the plate, whereas the volume increase in the wells with the added precision sphere is 298.5 nl. This boils down to a measured sphere volume of 290nl, which yields a measurement error of 22nl per well, and a measurement CV of 4.6 %.

Summary

This application note demonstrates the benefits of a volume measurement system like LUMINA, to track assay volumes over time, in order to investigate effects of evaporation or absorption on assay results.

It has been shown that assay plates filled with different volumes of water exhibit fast and easily measurable amounts of evaporation, whereas plates filled with DMSO are prone to water absorption. The relative measurement accuracy was evaluated with the help of precision spheres, which yielded a total error of only 22nl in average.

The LUMINA volume measurement system offers fast processing times (1536 wells in less than one minute) paired with an unmatched spatially resolved accuracy, which allows the system to be used in a variety of scenarios, including

- Inline process monitoring of assay volumes
- Inline detection of dispensing errors
- In- and offline calibration of dispensing and pipetting systems
- Evaporation control and subsequent backfill

Acknowledgements

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