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Measuring evaporation effects in high-throughput low volume assays in 1536 MWPs using LUMINA

Background

Liquid-based pharmaceutical and biological assays are critical tools in drug discovery, synthetic biology, and molecular diagnostics. Their success and reliability are highly dependent on the precise and accurate control of liquid volumes. With the ongoing push for higher throughput, assay miniaturization has become a standard, particularly with the adoption of 1536-well microplates. While miniaturization enables increased experimental density and reduced reagent consumption, it also introduces significant challenges—chief among them, evaporation effects and volume variability.

In these miniaturized formats, even minute deviations in liquid volumes can have disproportionately large impacts on assay performance. Variability in volumes can arise from several sources, including inaccuracies in liquid handling systems, prolonged dispensing times, inconsistent incubation conditions, and environmental factors such as humidity and temperature. One of the most insidious challenges, however, is evaporation during assay runs. Evaporation can lead to edge effects, where wells located on the periphery of the microplate experience greater volume loss compared to central wells. This spatial variability in volume can compromise assay results by altering solute concentrations, pH levels, and reaction kinetics, ultimately reducing reproducibility and reliability.

Volume deviations often remain undetected, as conventional quality control processes focus primarily on end-point assay performance rather than real-time monitoring of liquid volumes. This lack of visibility into liquid handling and evaporation dynamics underscores the urgent need for robust volume verification systems that can be seamlessly integrated into high-throughput workflows.

The LUMINA measurement system addresses this critical gap by offering an inline-compatible, optical technology capable of measuring absolute liquid volumes in 1536-well plates with high precision. Unlike other methods, LUMINA is designed to work with real assay fluids and is fully compatible with all standard high-throughput microplates. This compatibility ensures that the measurement process does not interfere with ongoing experiments, making it ideal for real-time monitoring and process control.

This study explores the application of the LUMINA system to quantify evaporation effects and volume variability in high-throughput, low-volume assays. By providing absolute volume measurements at the well level, LUMINA enables a detailed analysis of how factors such as plate geometry, assay fluid properties, and environmental conditions contribute to evaporation. For example, it was observed that peripheral wells in 1536-well plates are significantly more prone to evaporation compared to central wells, a phenomenon exacerbated by longer process times and higher temperatures.

The ability to measure and monitor liquid volumes in real-time not only enhances the reproducibility of high-throughput assays but also facilitates the optimization of assay designs and liquid handling protocols. These improvements are particularly valuable for biopharmaceutical applications, where data quality and reproducibility are paramount. Furthermore, the insights gained from volume measurement can inform the development of advanced laboratory automation systems capable of autonomously compensating for evaporation effects, thereby paving the way for more robust and reliable workflows in drug discovery and beyond.

Methods

The experimental procedure was conducted using Greiner Cellstar 1536 HiBase white plates (catalog number 782080), which are specifically designed for high-throughput applications and were used with lids to prevent evaporation during incubation. These plates provided the necessary well configuration for precise volume dispensing and measurements. In addition, Labcyte 1536 LP0400 plates were utilized for specific experimental conditions, ensuring compatibility with the dispensing system and overall experimental setup.

The reference liquid for the experiment consisted of two components: phosphate-buffered saline (PBS), which serves as a stable, isotonic solution to maintain cellular homeostasis, and a cell culture medium supplemented with fetal calf serum (FCS). The combination of PBS and

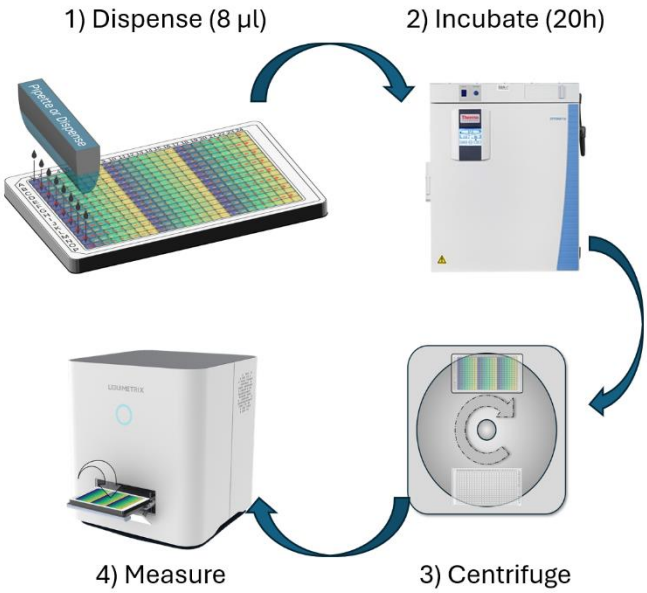

Figure 2: Measurement procedure using LUMINA.

FCS-containing medium allowed for the appropriate environment to support cell growth and functionality during the incubation period, providing the necessary conditions for the experiment.

To accurately dispense the reference liquid, the Thermo Multidrop dispensing system was employed. This system is known for its precision and efficiency in delivering small, reproducible volumes, which is critical for the high-throughput nature of the experiment. With the Multidrop, the required reference volume of 8 µl was delivered to each well of the plates. This precise liquid handling was essential for ensuring the consistency and reliability of the results.

For the incubation process, the experiment utilized varying incubator conditions, with an incubation time of 20 hours. The experiment was designed to test the effects of different environmental conditions, which could include temperature, humidity, and CO2 levels, depending on the specific incubator settings. This setup allowed for the investigation of potential variations in results under different incubation conditions.

Finally, volume measurements were taken immediately after the incubation period, which was crucial for determining the accuracy and potential changes in liquid volume within each well. The timing of the measurements ensured that any evaporation or changes in volume could be accurately recorded and accounted for, maintaining the integrity of the experimental data. The precise measurement technique was necessary for ensuring that any observed effects could be attributed to the experimental conditions rather than inconsistencies in the liquid handling process.

Results

The factors influencing liquid volumes during the experiment were carefully evaluated, with several key variables identified as having a significant impact on volume stability and accuracy. One primary factor was the plate effects, which include variations in well geometry and the material properties of the plates. Differences in the design and surface characteristics of the wells can lead to slight inconsistencies in liquid handling, such as how the liquid adheres to the well walls or how it evaporates. These plate effects can introduce variability into the results, making it essential to account for them when analyzing the data.

Another important factor was dispensing variations, which refer to differences in the accuracy and precision of the liquid dispensing process. Even small deviations in the volume delivered by the dispensing system can accumulate, leading to discrepancies in the final volume in each well. The use of precise dispensing systems, such as the Thermo Multidrop, is crucial for minimizing these

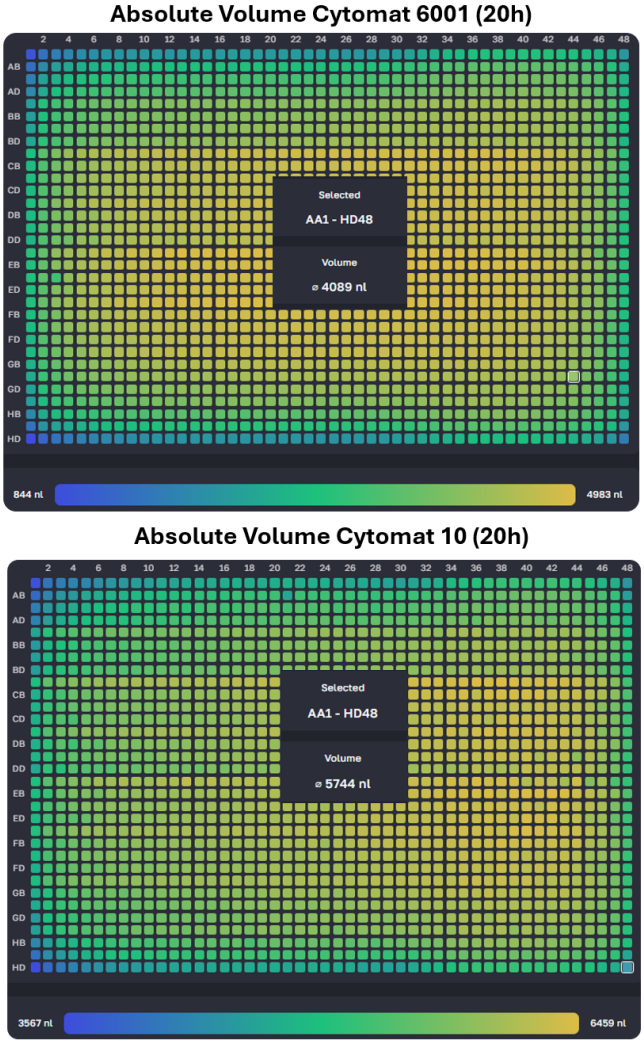

Figure 1: Comparison of incubation effects in Cytomat 6001 and 10, using identical liquid handling procedures

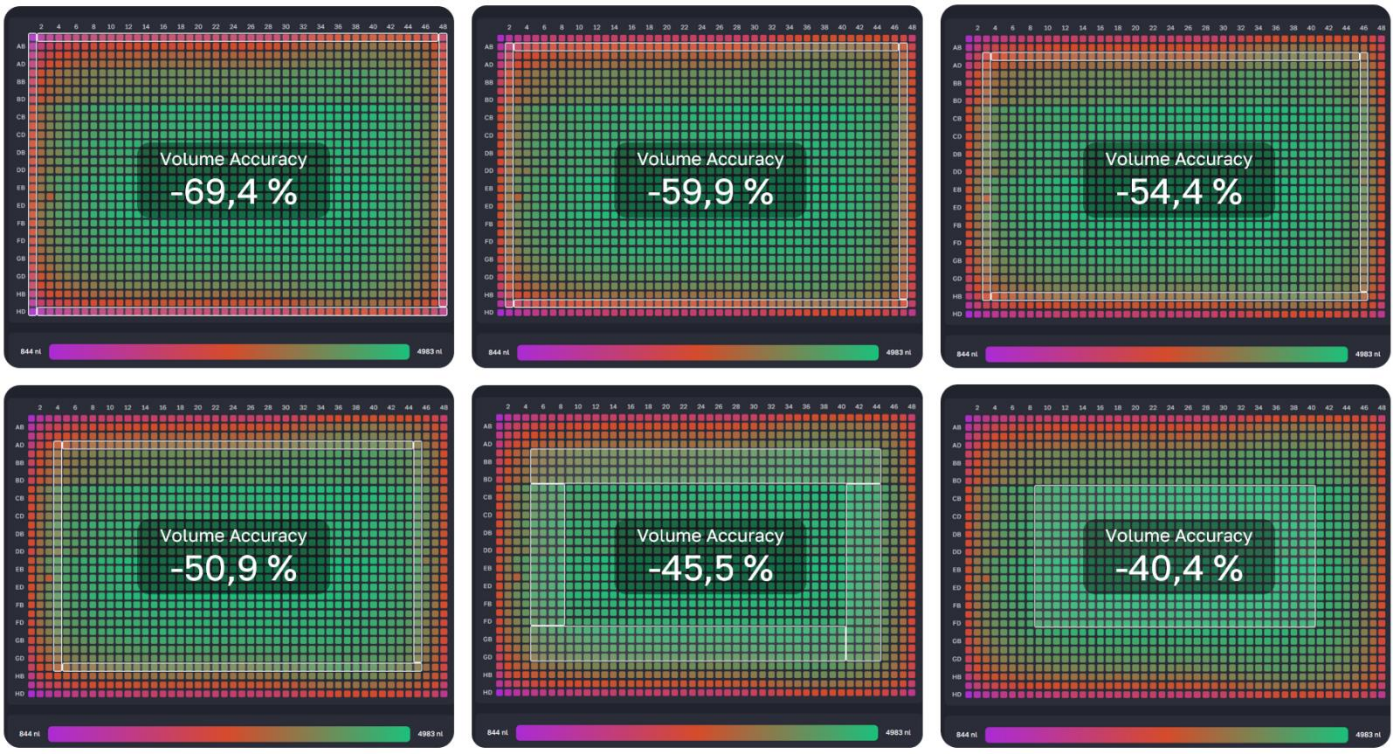

Figure 3: Evaporation effects across various well zones (white selection); accuracy compared against the 8 µl reference

variations, but slight differences can still arise due to equipment calibration, liquid viscosity, or other factors.

Sealing methods also played a critical role in the experiment. The use of lids or sealing films can significantly affect the volume stability by reducing evaporation. However, the choice of sealing method can also influence the extent of evaporation. For instance, some lids or films might offer better sealing properties than others, thereby providing a more stable environment for the liquid during incubation. In contrast, insufficient sealing can lead to increased liquid loss due to evaporation, especially over extended incubation times.

The incubation conditions further impacted liquid volumes. Variations in incubator type, incubation method, and the positioning of the plates within the incubator contributed to differences in temperature, humidity, and airflow around the plates. These environmental factors can influence evaporation rates and, therefore, the final volume measurements. For example, plates placed in different locations within the incubator may experience slightly different temperatures or humidity levels, which can affect how much liquid evaporates.

Finally, the liquid type itself was an important consideration. The composition of the liquid, including its viscosity, surface tension, and other chemical properties, can influence its evaporation rate. Liquids with higher water content tend to evaporate more quickly, while those with higher concentrations of solutes or proteins may be less prone to evaporation. The use of PBS and cell culture media with FCS in the experiment presented specific challenges in this regard, as these liquids have varying evaporation rates depending on their composition.

In terms of evaporation insights, the experiment revealed that overall liquid evaporation could be effectively quantified using LUMINA, which provided valuable data on the extent of volume loss across the wells. Specific evaporation effects were detected, with higher evaporation rates observed in the edge wells. This is a common phenomenon in high-throughput experiments, where the wells at the edges of the plate are more exposed to air and often experience more significant temperature fluctuations.

Moreover, evaporation patterns were found to be significantly influenced by incubator settings, demonstrating how critical it is to control environmental conditions. Variations in temperature, humidity, and airflow in the incubators could cause uneven evaporation across the plate, further emphasizing the importance of consistent incubation conditions for reliable volume control. This insight highlights the need for standardized incubation protocols and suggests that careful attention to environmental factors is essential for minimizing evaporation and ensuring accurate and reproducible results.

Conclusion

The use of LUMINA for volume control significantly enhances assay workflows by optimizing several critical aspects of the process. One key advantage is its ability to calibrate dispensers with specific assay liquids, ensuring that the exact volumes are delivered accurately to each well, regardless of the liquid's properties. This precise calibration is essential for achieving consistent and reliable results in assays.

Additionally, LUMINA aids in selecting the most appropriate sealing methods for extended incubation periods. Proper sealing helps to prevent evaporation, ensuring that the liquid volumes remain stable throughout the incubation phase. This step is crucial for maintaining the integrity of the assay and for minimizing any potential sources of error related to volume changes.

Another important feature of LUMINA is its ability to exclude specific edge wells from analysis. Edge wells are often subject to temperature and evaporation variations, which can skew results. By excluding these wells from the analysis, LUMINA improves the overall accuracy of the data, ensuring that only the most reliable measurements are included.

LUMINA also supports the backfilling of evaporated liquid, which is essential for maintaining assay integrity, especially in long-duration incubations. This feature helps to replenish lost volume and ensures that the assay conditions remain consistent, preventing any unwanted variability that could affect the results.

Finally, LUMINA provides long-term insights into lab workflows, allowing for continuous process improvement. By identifying patterns in volume deviations over time, researchers can make informed decisions to further optimize their workflows, leading to improved efficiency and more accurate assay outcomes.

Overall, LUMINA serves as a robust inline control tool for identifying and mitigating volume deviations, ultimately enhancing the precision and reproducibility of assays. Its comprehensive approach ensures that the assay process remains efficient, reliable, and consistent, contributing to better research outcomes and more reliable data.

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