In collaboration with

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Accurate Compensation of Evaporation Effects in Microtiter Plates Using LUMINA and CERTUS FLEX

Introduction

The evaporation of liquids in microtiter plates during prolonged incubations is a well-documented challenge in cell culture and assay workflows. Evaporation alters the concentrations of dissolved substances, such as salts, nutrients, and supplements, leading to changes in osmolarity and pH. Such changes can significantly impact cell behavior, including growth, metabolism, and response to stimuli. Addressing this challenge requires precise measurement and compensation of evaporation-induced volume loss.

Furthermore, accurate liquid handling is critical in workflows like plate-based single-cell RNA-sequencing, where cDNA concentration normalization is essential for sequencing depth consistency.

The LUMINA device offers a breakthrough solution by enabling nanoliter-scale volume measurements for each well in a microtiter plate. Combined with a precision dispenser like the CERTUS FLEX from nNano, LUMINA facilitates targeted volume restoration to compensate for evaporation effects.

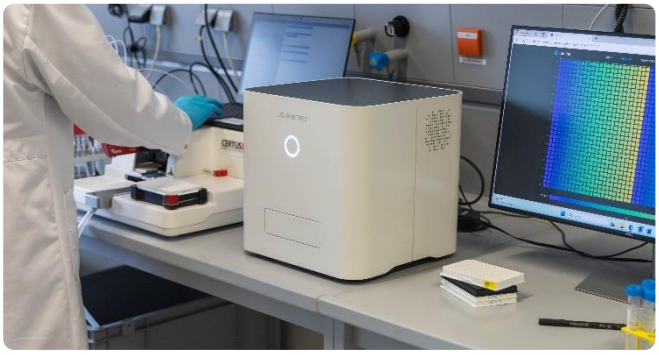


Figure 1: Setup with CERTUS FLEX and LUMINA

Impact of Evaporation on Cell Cultures

Evaporation in microtiter plates is not uniform. Edge wells typically experience greater volume loss due to their increased exposure to air, known as the “plate effect.” This differential evaporation leads to variability in cell culture conditions, impacting:

- **Cell growth**: Changes in osmolarity can inhibit or promote cell proliferation.
- **Metabolic activity**: Altered concentrations of nutrients and salts can affect metabolic pathways.
- **Experimental reproducibility**: Variability in well volumes undermines assay consistency and reliability.

Experimental Setup

To demonstrate the capability of LUMINA in addressing evaporation effects, we conducted a controlled experiment using a 1536-well microtiter plate. The plate was filled with 8 µl of liquid per well using a single dispensing channel. After a 10-hour incubation without a lid, evaporation was measured and compensated as follows:

Initial Measurement

- Directly after dispensing, the LUMINA device measured the initial volume in each well to establish a baseline.
- Data confirmed uniform dispensing of 8 µl across all wells (Figure 2).

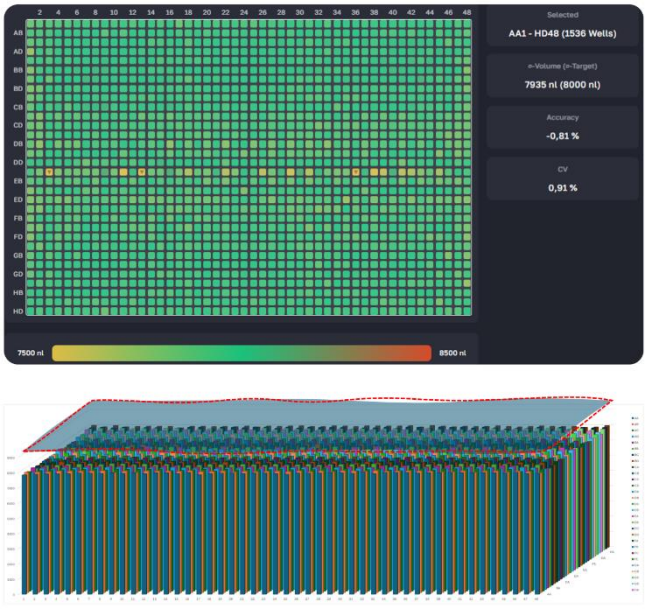


Figure 2: 1536 MWP filled with 8µl (ACC -0.81%, CV 0.91%)

Evaporation Phase:

- The plate was left uncovered for 10 hours at room temperature to allow for natural evaporation.
- LUMINA measurements were repeated to quantify the volume loss in each well (Figure 3).

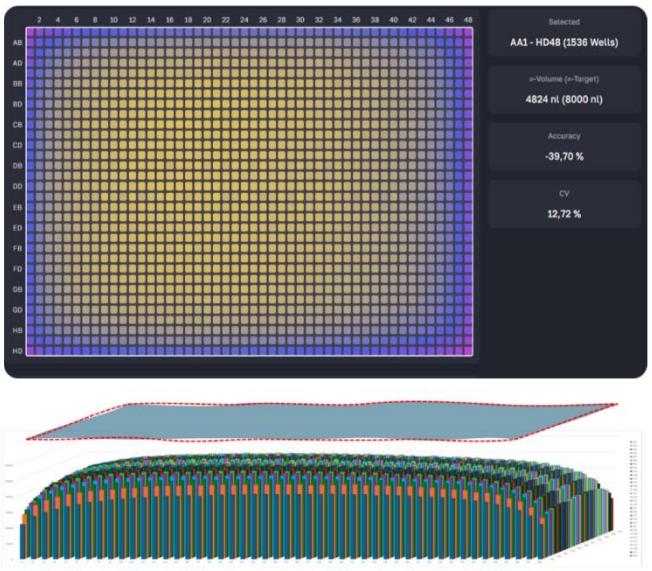


Figure 3: Volume distribution after 10h evaporation at room temperature

Compensation Phase

The volume difference between the target (8 µl) and the measured volume after evaporation was calculated for each well. This data was used to program the CERTUS FLEX dispenser, equipped with four LUMINA-calibrated channels (Figure 4), to refill each well precisely to 8 µl. The refill process restored uniform volume across all wells (Figure 5).

The calibration of the CERTUS FLEX dispenser using LUMINA is described in detail in **Application Note #2503.**

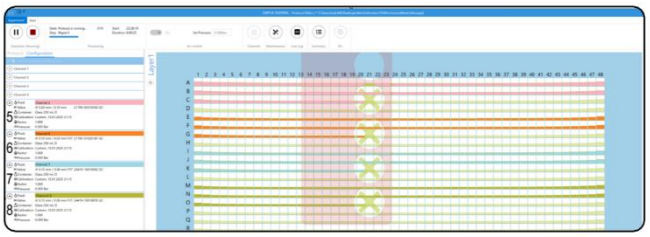


Figure 5: Channel backfill process on CERTUS FLEX

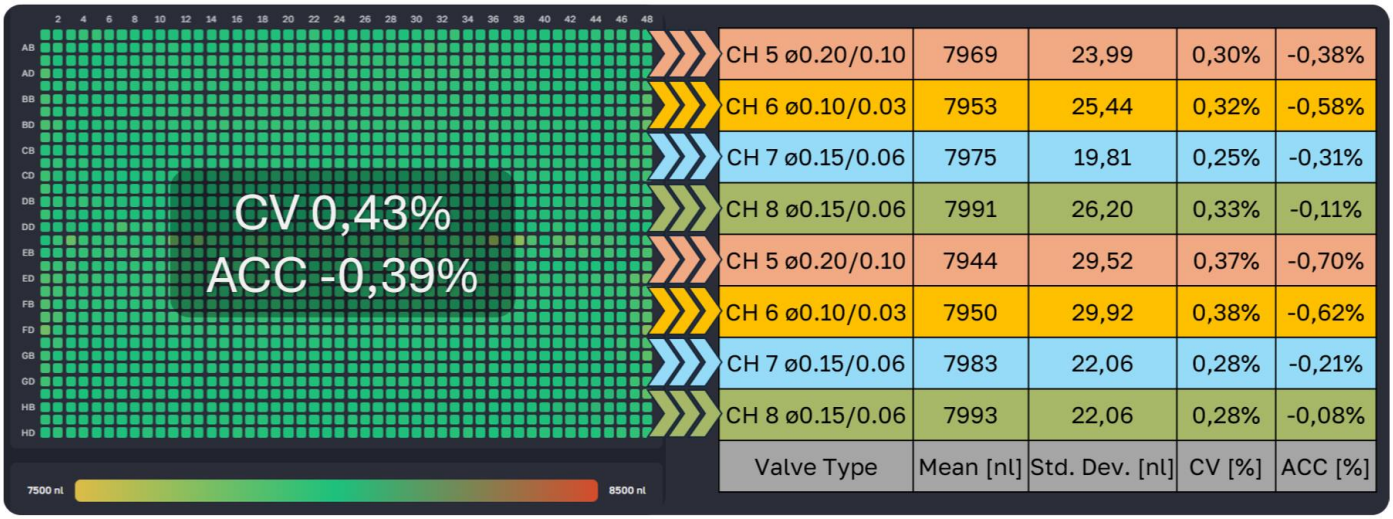
Results

LUMINA's nanoliter-scale accuracy enabled precise quantification of evaporation patterns, with edge wells exhibiting the highest volume loss. Following targeted refilling using the CERTUS FLEX dispenser, the variability in well volumes was eliminated, demonstrating

- Accurate volume measurement: LUMINA reliably quantified evaporation-induced volume differences.
- Precise dispensing: The CERTUS FLEX dispenser, calibrated with LUMINA data, restored well volumes with highest accuracy.

Table 1: Comparison of the weighed and measured well volumes for each process step

	Weight (mg)	Liquid / Net **Weight** (mg)	Weighed Avg. **Well Volume** (nl)	Meas. Avg. **Well Volume** (nl)	**Difference** (nl)
Empty	40424	-	-	-	-
Filled	52637	12213	7951	7935	**16**
Evaporated	47859	7435	4840	4824	**16**
Refilled	52678	12254	7978	7969	**9**



CH 5 ø0.20/0.10	7969	23,99	0,30%	-0,38%
CH 6 ø0.10/0.03	7953	25,44	0,32%	-0,58%
CH 7 ø0.15/0.06	7975	19,81	0,25%	-0,31%
CH 8 ø0.15/0.06	7991	26,20	0,33%	-0,11%
CH 5 ø0.20/0.10	7944	29,52	0,37%	-0,70%
CH 6 ø0.10/0.03	7950	29,92	0,38%	-0,62%
CH 7 ø0.15/0.06	7983	22,06	0,28%	-0,21%
CH 8 ø0.15/0.06	7993	22,06	0,28%	-0,08%
Valve Type	Mean [nl]	Std. Dev. [nl]	CV [%]	ACC [%]

Figure 4: Accuracy & CV of the refilled 1536 MWP – each well refilled with individual evaporated volume

Conclusion

This application note demonstrates the combined power of LUMINA and the CERTUS FLEX dispenser in mitigating evaporation effects in microtiter plates. The ability to precisely measure and compensate for volume loss ensures consistent experimental conditions, improving the reliability and reproducibility of cell-based assays. These results underscore the potential of LUMINA as a critical tool for laboratory automation and high-throughput screening.

Future Directions

The integration of LUMINA's measurement capabilities with automated workflows opens avenues for:

- Dynamic evaporation monitoring during long-term incubations.
- Real-time compensation strategies in high-throughput screening setups.
- Enhanced assay precision across diverse plate formats and experimental conditions.

Acknowledgements

We thank nNano for providing the CERTUS FLEX dispenser and technical support. For additional information, please contact **sales@nnano.com** or visit **www.nnano.com**

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