In collaboration with

uk robotics

UK Robotics – 267 Bolton Road, Westhoughton, BL5 3EH, UK

Concentration Normalization

Accurate compensation of evaporation effects In microtiter plates using LUMINA and D2

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 D2 from UK Robotics, LUMINA facilitates targeted volume restoration to compensate for evaporation effects.

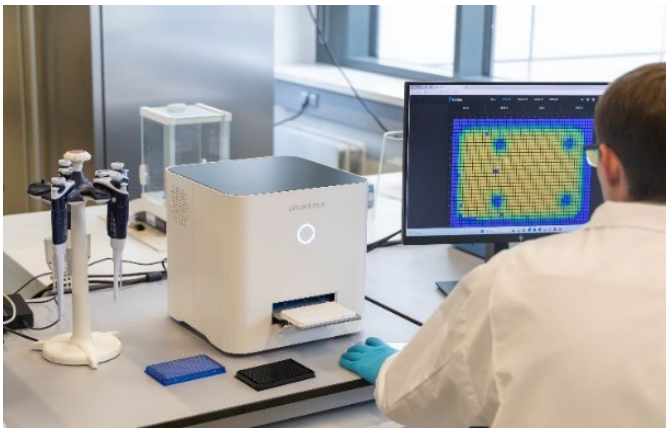

Figure 1: The LUMINA system in use

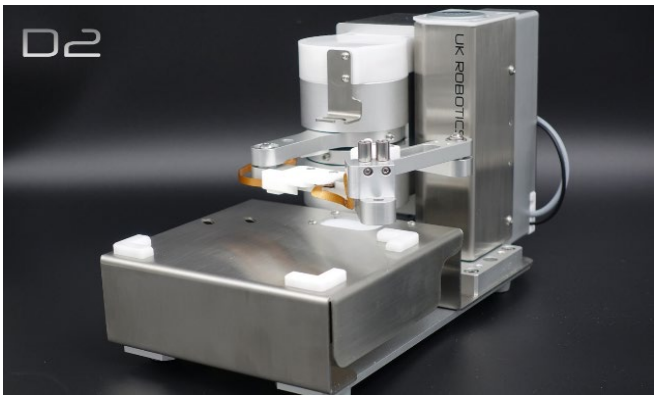

Figure 2: UK Robotics D2 dispenser

Impact of evaporation on Cell culture

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-wellmicrotiter plate. The plate was filled with 5 µl of liquid per well using UK Robotics D2. After a 2.5-hour incubation period at ambient temperature (20°C) without a lid, evaporation was measured and compensated as follows:

Initial Measurement

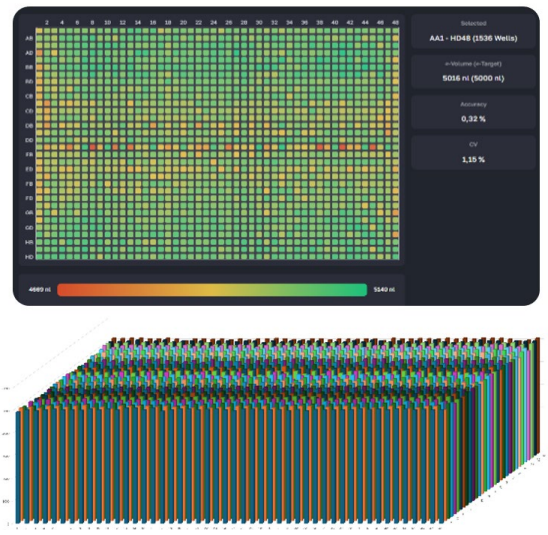

Figure 3: 1536 MWP filled with 5µl
(Accuracy -0.3 %, CV 1.2 %)

- Directly after dispensing, the LUMINA device measured the initial volume in each well to establish a baseline.
- Data confirmed uniform dispensing of 5 µl across all wells (Figure).

Evaporation Phase

- The plate was left uncovered for 2.5 hours at room temperature to allow for natural evaporation.
- LUMINA measurements were repeated to quantify the volume loss in each well (Figure).

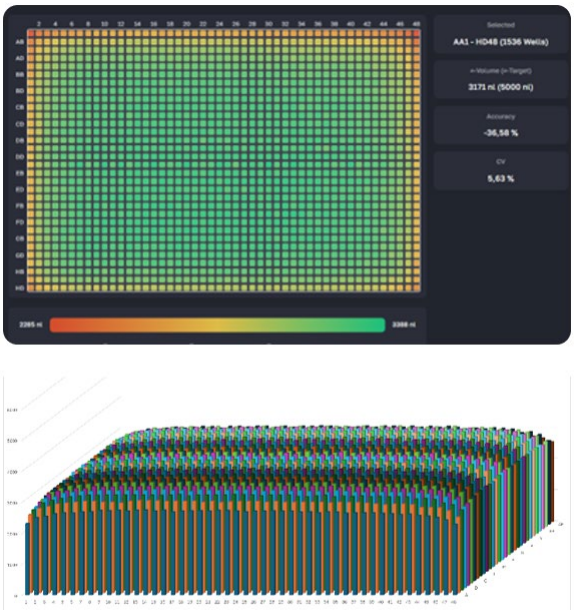

Figure 4: Volume distribution after 2.5h at RT (CV 5.6 %)

Normalization Phase

The volume difference between the target (5 µl) and the measured volume after evaporation was calculated for each well. This data was used to program the D2 dispenser to refill each well precisely back to 5 µl (Figure).

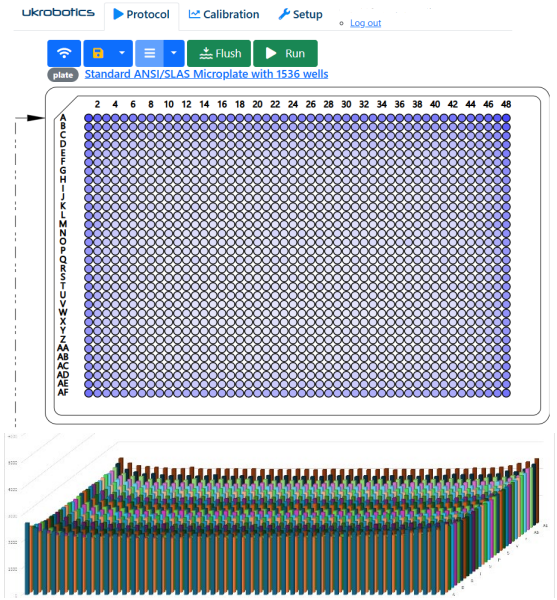

Figure 5: D2's normalization protocol (max dispense volume 2.7 µl)

The refill process restored uniform volume across all wells with even lower CV than the original fill (Figure).

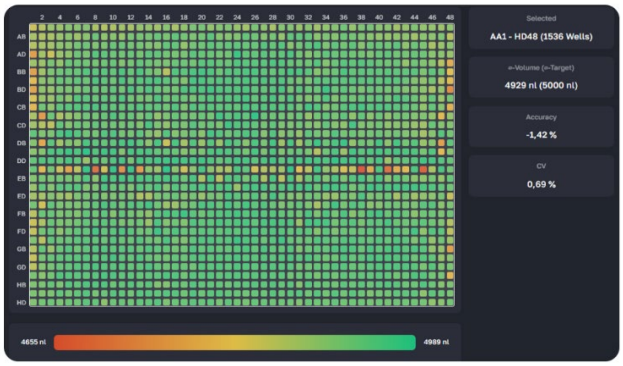

Figure 6: Volume distribution after normalization (Accuracy 1.4 %, CV 0.7 %)

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 D2 dispenser, the variability in well volumes was eliminated, demonstrating:

- **Accurate volume measurement**: LUMINA reliably quantified evaporation-induced volume differences.
- **Precise dispensing**: The D2 dispenser restored well volumes to their original values, thus normalizing concentrations across the plate

Conclusion

A This application note demonstrates the combined power of LUMINA and the D2 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 Direction

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

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

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