

Characterization and mitigation of Evaporation in the Reshape Imaging Device

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Introduction

Evaporation is a common challenge in various biological assays, particularly those involving small volumes or elevated temperatures. As liquid evaporates over time, systematic errors may arise, compromising data integrity. Volume reduction due to evaporation affects the concentration and osmolarity of reagents, with these effects becoming increasingly significant as assay duration extends and volume decreases.

This issue is particularly pronounced in assays running for several days at high temperatures (>37°C), where evaporation can introduce significant inconsistencies and potential misinterpretations. In biological assays, the primary consequences of evaporation include:

- **Volume Reduction:** As water evaporates, solute concentrations, including salts, proteins, and enzymes, increase, potentially skewing results—especially in enzyme kinetics and colorimetric assays.
- **pH Alterations:** Many assays depend on pH-sensitive reactions. Evaporation can disrupt buffer composition, affecting enzymatic activity and reaction specificity.
- **Edge Effects in Microplates:** In high-throughput screening using microplates, wells at the periphery experience greater evaporation than those in the center, leading to inconsistent reaction conditions and reduced reproducibility.
- **Temperature-Dependent Evaporation:** Higher incubation temperatures accelerate evaporation, exacerbating concentration discrepancies—particularly in PCR and ELISA-based assays.

Addressing evaporation is crucial for enhancing assay robustness. Minimizing evaporation at lower volumes will also improve assay scalability.

This study investigates evaporation in the Reshape imaging device, used for biological and biotechnological assay optimization, to ensure evaporation remains minimal and does not interfere with assay performance.

The Reshape imaging device is capable of holding 15 petri dishes or 10 microtiter plates.

Different hardware versions (denoted 3.0a, 3.5b etc) were included systematically in the trial to document the homogeneity of performance across hardware versions.

Materials and Methods

Evaporation Homogeneity Assay

Methyl violet (0.1% w/w) was used as a colorimetric agent to track evaporation. As it evaporates, the wells go from a deep blue initially to clear upon total evaporation, allowing individual automatic measurement of when all the liquid in a well has evaporated through a colorimetric measurement. Based on the time to total evaporation and assuming linear evaporation rates, the average evaporation rate (volume/time) was calculated per well. If not denoted, the starting volume for each well was 50 μ l.

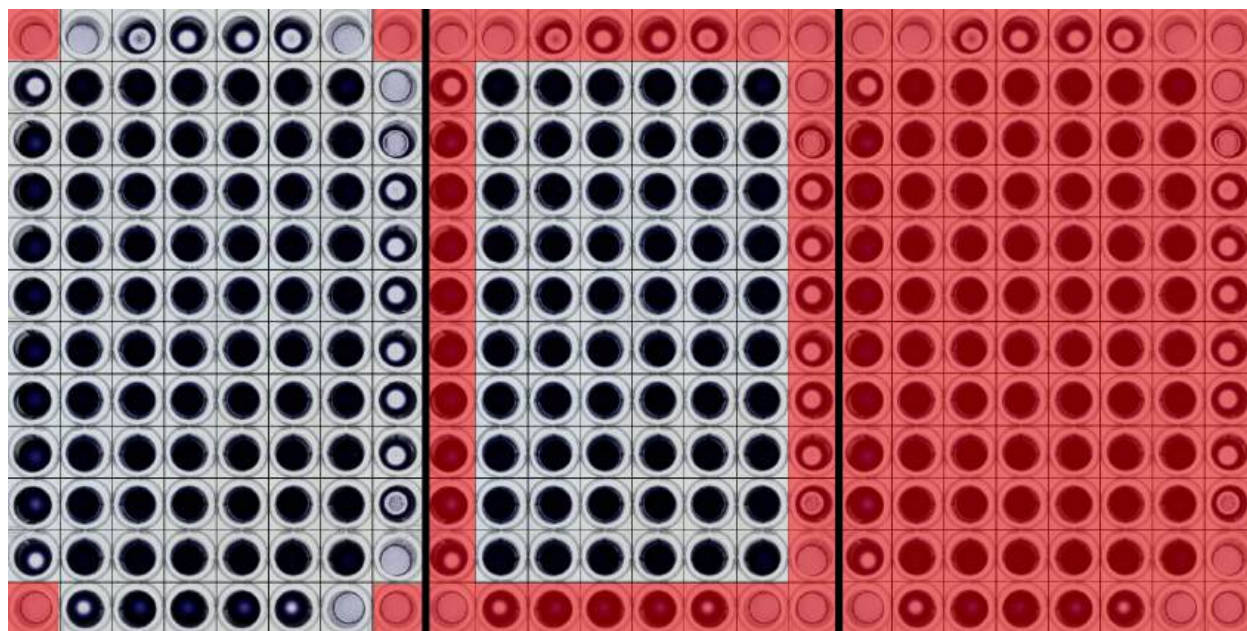


Figure 1: Left showing corner wells for a single plate marked with transparent red color, middle showing perimeter wells, right showing the entire plate.

Evaporation characteristics were analyzed for the subsets of wells (corner wells, perimeter wells, and the entire plate). 10x 96-well plates were run in parallel in the imaging device - the standard deviation reported is the standard deviation for each subset of wells across the 10 individual 96-well plates.

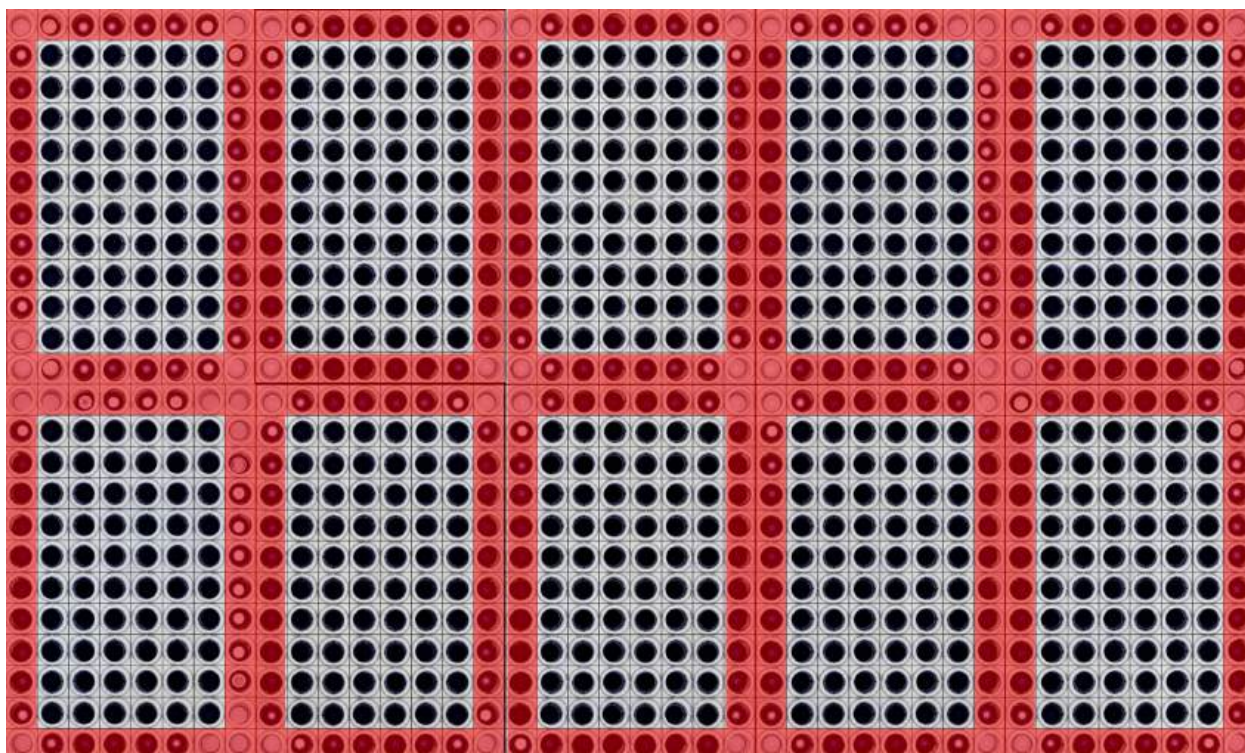


Figure 2: A full Reshape imaging device, in this case with perimeter wells marked for all 10 plates. Each plate was considered a technical replicate and used to calculate homogeneity as the variability between each replicate using standard deviation.

Standard deviation was calculated for the average value of technical replicates (**N = 10**). For specific analyses:

- **Corner wells:** Stdev was calculated across selected wells (A1, A12, H1, H12).
- **Perimeter wells:** Stdev was calculated across all perimeter wells in each plate.
- **Whole plate:** Stdev was calculated across all wells in each plate.

All measurements were normalized by subtracting initial values at t=0.

Evaporation assays were performed at three temperatures:

- Room temperature (~25°C)
- 30°C
- 37°C

Total Evaporation Assay

Plates were prepared as in the Evaporation Homogeneity Assay, but instead of individual colorimetric well assessments, the whole plates were weighed at certain timepoints, allowing analysis of total plate evaporation.

Results

Evaporation Homogeneity Assay

Corner well analysis

Corner wells dried out completely at 30°C and 37°C, and so could be used to accurately estimate an average evaporation rate:

- **At ambient temperature (~25°C):** Complete dry-out of corner wells was not observed within the experiment's duration (last measurement at 120 hours)
- **At 30°C:** Complete dry-out of the corner wells occurred after 110 hours, equivalent to evaporation in the corner wells of 0.45 ul/hour
- **At 37°C:** Complete dry-out of the corner wells occurred after 76 hours, equivalent to evaporation in the corner wells of 0.66 ul/hour

Perimeter well analysis

The perimeters with 50ul were not dried out after 120 hours, and so it was not possible to analyse a time to evaporation or calculate an average evaporation rate. Figure 6 shows an example set of 10 plates at 120 hours for reference.

We estimate qualitatively that the perimeters have an average dry-out rate that is half of the corners based on the data presented in figure 3:

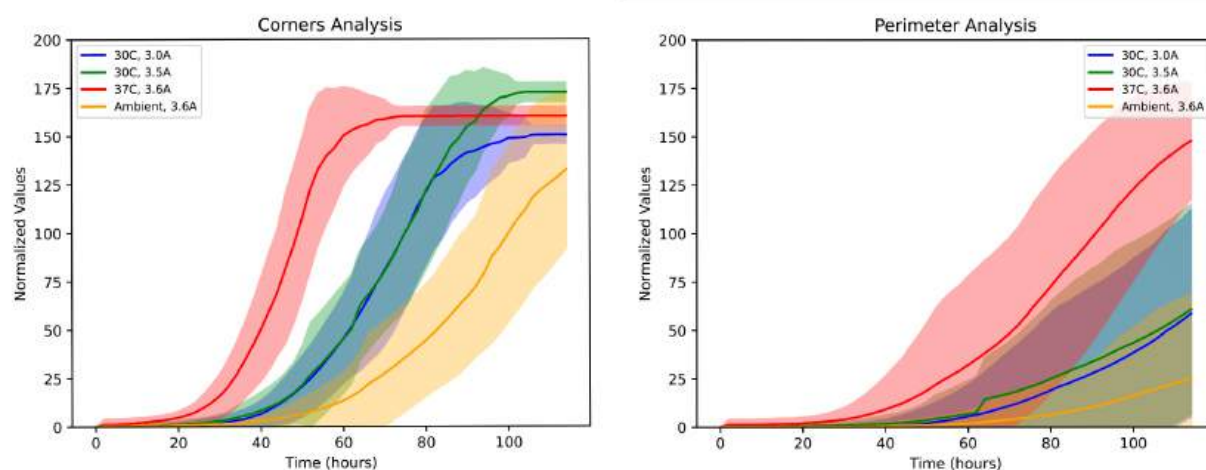


Figure 3: Colorimetric, normalized values (HSV) of corners relative to perimeters over time (main line is the average with error bar indicated by lighter colored area)

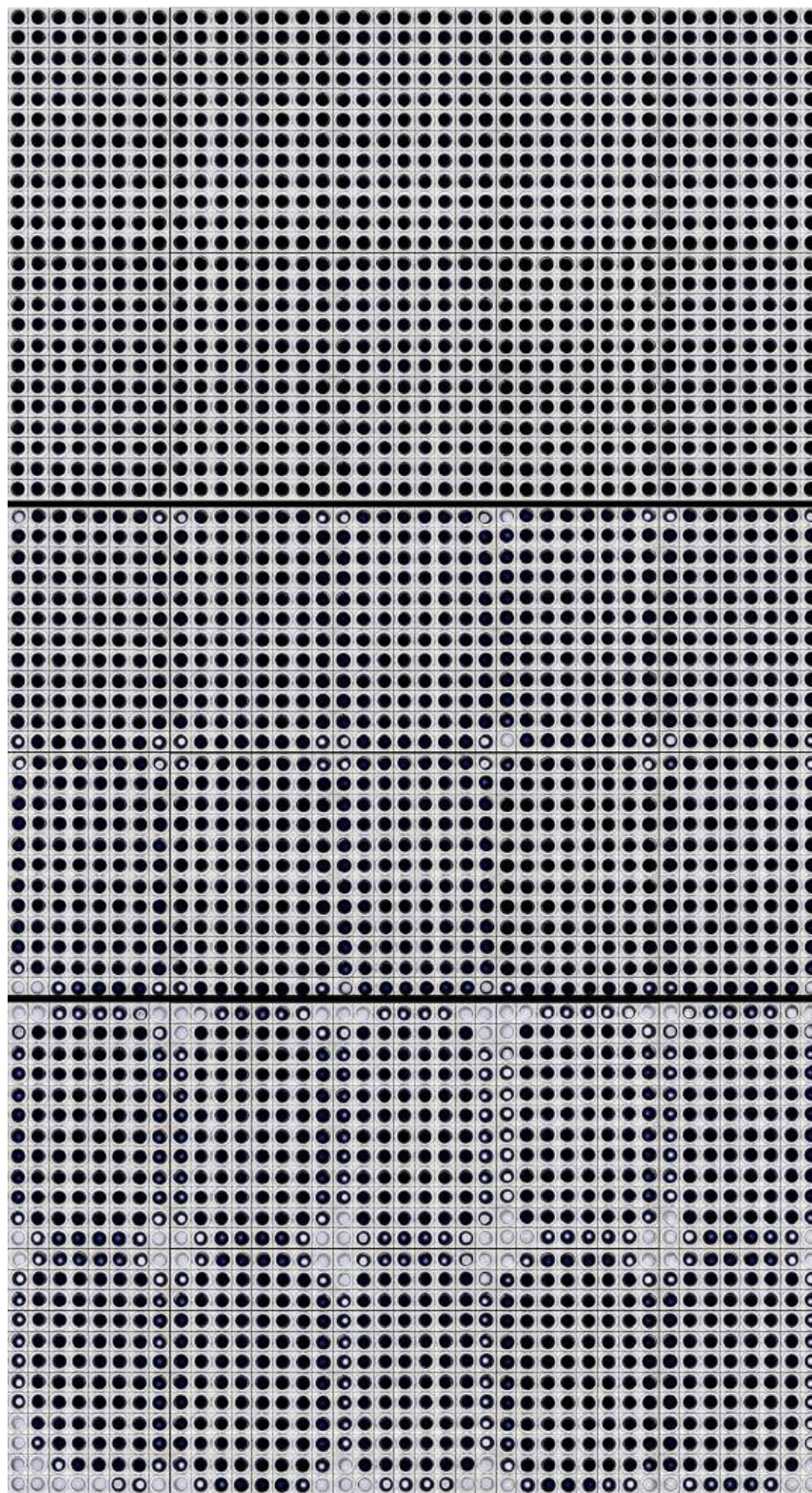


Figure 4: Top image is a full set of plates imaged at timepoint 0, in the middle a full set of plates imaged at timepoint 72 hours and at the bottom imaged again at 120 hours.

Total evaporation rate

Total evaporation rates and metadata were summarized in **Table 1**.

Table 1: Summary of Total Evaporation Rates

Temperature	Plate format	Initial volume per well	Duration of assay	Average well dry-out rate	Percent evaporation per well per hour	Hardware version	Relative humidity
37	90mm petri	10 ml	112 hours	41.9 ul/hour	0.42%	3.5a	Estimated at ~23%
25	90mm petri	10 ml	112 hours	13.69 ul/hour	0.14%	3.5a	Estimated at ~23%
30	12 well plate	1 ml	112 hours	1.64 ul/hour	0.16%	3.5a	Estimated at ~23%
37	96 well plate	100 ul	42 hours	0.47 ul/hour	0.47%	4.0b	23% avg relative humidity
37	96 well plate	100 ul	42 hours	0.33 ul/hour	0.33%	4.0b	47% avg relative humidity

BINDER incubator compared to Reshape imaging device

A side-by-side experiment was conducted with the same plates prepped and placed into a Reshape imaging device (v3.6a) at 37 degrees and a Binder BF260 incubator at 37 degrees, 80% fan speed. A single image was taken at 64 hours, showcasing the overall evaporation homogeneity and rate when comparing the two systems.



Figure 5: Parallel setup with the plates in the Binder incubator

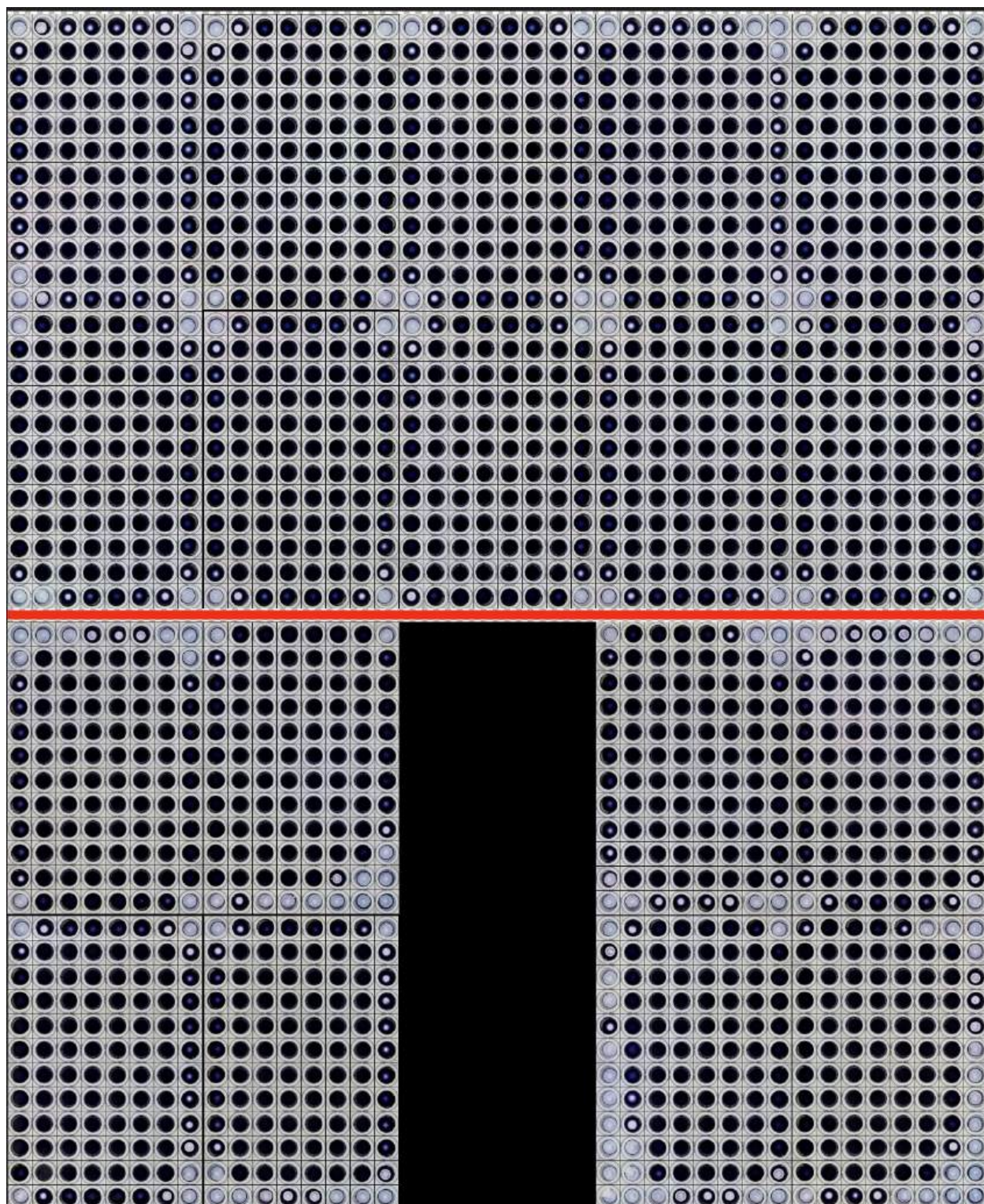


Figure 6: At the top, the plates that had been incubated in the Reshape imaging device for 64 hours. On the bottom, the plates that had been incubated in the Binder incubator for 64 hours.

Discussion and mitigation

The evaporation rates in this study were entirely based around dilute methyl violet solutions in water. Agar-based plates were not quantified for evaporation rates, but would likely slightly reduce the evaporation rates¹.

Based on the results presented, suitable assessments can be made on the need for mitigation of evaporation-based loss of volume in wells.

Thermofisher [Nunc Edge plates](#) were not tested but would provide about a 10x decrease in evaporation rates (based on the Thermofisher application note available) while the “moat” is filled with water, which can be a key tool for long-running assays or assays sensitive to evaporation/concentration changes. The evaporation rates found for commodity 96 well plates were in line with those described in the application note for Nunc Edge plates.

Passive evaporation from containers in the chamber allowed us to increase relative humidity from 23% to 47%, which caused a 30% decrease in evaporation rates in 96 well plates at 37 degrees C. Allowing open containers with water to sit passively in the chamber can be a simple tool to decrease evaporation rates.

Sealing the plates with parafilm or tape may be a suitable mitigation strategy for preventing evaporation. This was not tested in the study.

In certain cases, not utilizing the corner wells or even the perimeter wells may be suitable as these are by far the most sensitive to evaporation. Simply filling them with water will mitigate most of the evaporation in the inner wells of the plate, similar to the Nunc Edge plates reference above.

Conclusion

The Reshape imaging device product was shown to show similar or less evaporation with better homogeneity than a regular incubator (Binder BF260).

It was demonstrated that the hardware version of the imaging device did not have a significant impact on the evaporation rates measured.

This study confirms that **temperature is the key factor in evaporation rates**, with significant volume loss observed at higher temperatures.

Mitigating evaporation is crucial for assays requiring precise quantitative outputs. Future research should explore new techniques to **limit evaporation or better control it**, especially in high-throughput and microscale settings.