APPLICATION NOTE

Robust 3D spheroid screening of ADCs and biologics with the FlexDrop Plus non-contact dispenser.

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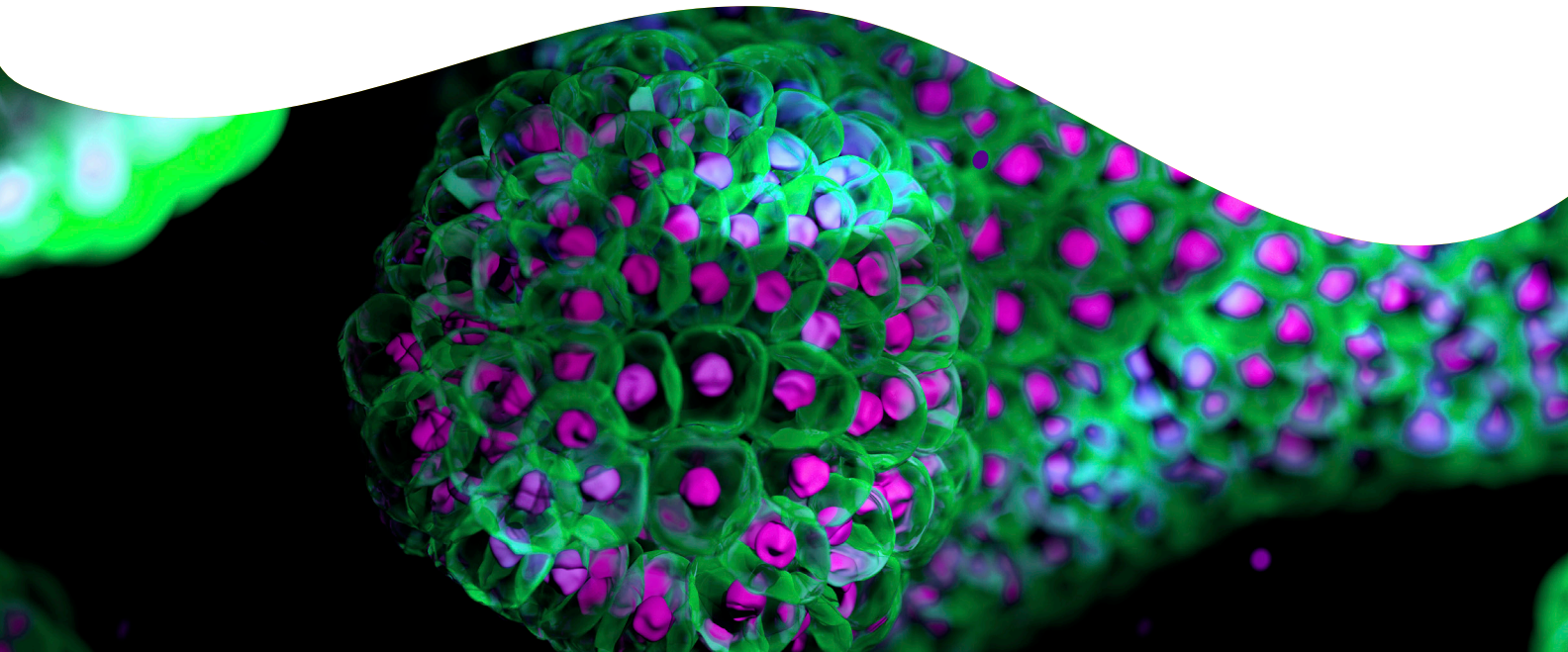
Introduction

Antibody–drug conjugates (ADCs) are an increasingly important class of targeted cancer therapeutics, combining the selectivity of monoclonal antibodies with the delivery of highly potent cytotoxic payloads to target-expressing cells. This targeted delivery can enhance tumor specificity while reducing off-target effects. As ADCs become more prevalent in drug development pipelines, there is a growing need for robust and predictive *in vitro* screening approaches capable of accurately assessing their biological activity alongside other therapeutic modalities, including monoclonal antibodies and small molecules (Lyons et al., 2021; Tsuchikama et al., 2024).

A key challenge in screening biologics such as ADCs is their physical complexity. Higher solution viscosity, susceptibility to shear-induced denaturation, and propensity for aggregation can adversely affect dispensing performance, contributing to variability in transfer volume and reduced reproducibility at the low volumes required for miniaturized assays (Yang et al., 2021).

Alongside these dispensing challenges, there is a growing shift toward three-dimensional (3D) *in vitro* models such as spheroids, which more accurately replicate tumor architecture, microenvironmental gradients, and cell–cell interactions compared to traditional monolayer cultures. These features are particularly relevant for ADCs, where efficacy depends on cellular internalization, intracellular processing, and bystander effects that rely on payload diffusion through tissue-like structures (Barbosa et al., 2021).



The Revvity FlexDrop™ Plus Non-Contact Dispenser is designed to address these challenges, enabling accurate low-volume dispensing of challenging reagents including viscous protein formulations. Using positive-pressure dispensing with independently controlled channels, customizable liquid classes, and integrated droplet verification, FlexDrop Dispenser supports consistent and reproducible transfer across a broad range of liquid types and formulations (Revvity, 2025).

In this study, we evaluate the performance of FlexDrop dispenser relative to acoustic dispensing for the delivery of ADCs, monoclonal antibodies, and small molecules into 3D spheroid assay formats. We assess assay robustness, reproducibility, spheroid integrity, and downstream pharmacological responses to demonstrate how optimized liquid handling can support high-quality screening data from complex biological systems.

Methods

The performance of the FlexDrop dispenser was evaluated relative to acoustic dispensing using small molecules, monoclonal antibodies, and antibody–drug conjugates (ADCs) in 3D spheroid screening assays. All studies were performed by the Revvity Preclinical Services.

Cells were seeded into 384-ultra-low attachment microplates and cultured for 24 h to allow spheroid formation prior to dosing. Compounds were delivered using either the FlexDrop dispenser or acoustic dispensing, followed by a 96 h incubation period before assay readout.

Assay robustness was evaluated across five cell lines using four classes of combination treatment:

1. Small molecule × Small Molecule (SM × SM)
2. Monoclonal Antibody × Small Molecule (mAb × SM)
3. Small Molecule × ADC (SM × ADC)
4. ADC × monoclonal antibody (ADC × mAb)

Assay quality was assessed using Z-factor and coefficient of variation (%CV) analyses. Data processing, visualization, and statistical analyses were performed using Signals Spotfire® software.

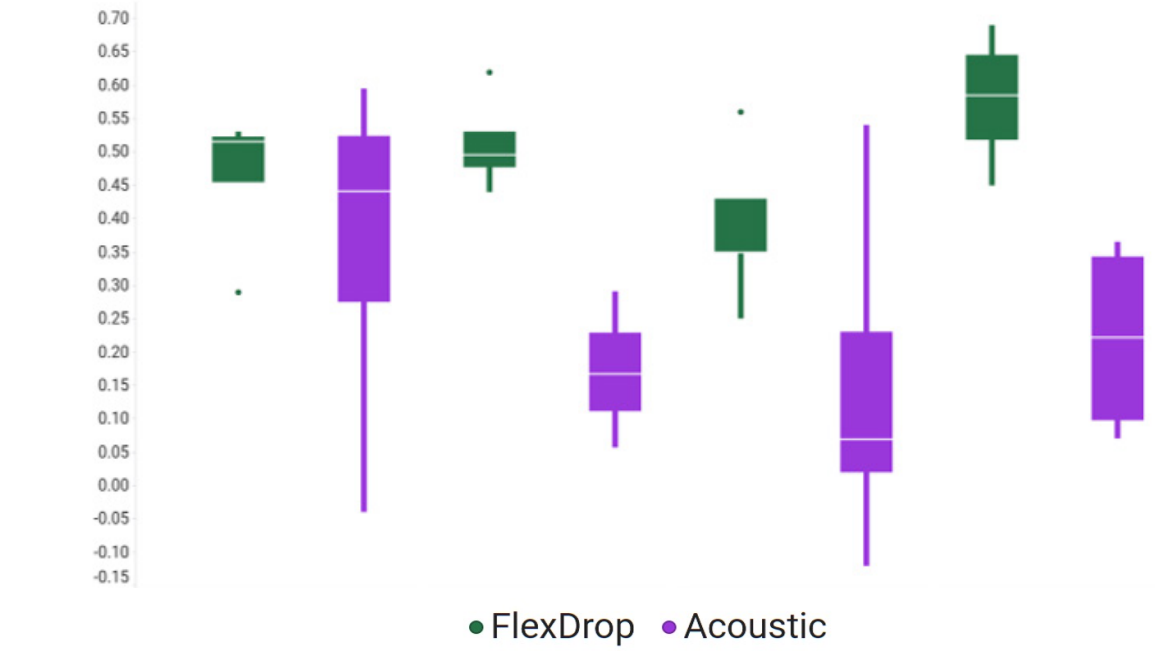
Pharmacological performance was evaluated in SW948 3D spheroid cultures using SN-38 and Labetuzumab Govitecan (LG). Labetuzumab Govitecan contains an SN-38 payload; therefore, the SN-38/LG combination was selected as a representative small-molecule/ADC dispensing model rather than as an evaluation of mechanistically distinct combination therapy. This pairing provided a biologically relevant model system in which the same cytotoxic payload was evaluated both as a free small molecule and as an ADC, enabling assessment of dispensing performance across distinct reagent classes within a single screening workflow. Single-agent dose–response profiles and combination inhibition responses were derived from the same experimental dataset. Cell viability was quantified using the ATPlite™ 1 glow-F luminescence assay.

To assess the impact of dispensing on 3D spheroid morphology, A549 spheroids treated with vehicle controls (DMSO or PBS) were imaged 96 h post-dosing using the Opera Phenix™ High-Content Screening System.

Results

Robust and reproducible assay performance

Assay robustness and dispensing performance was evaluated in 3D spheroid cultures across five cell lines using four classes of combination treatment (Figure 1). These treatment classes were selected to represent the range of reagent types commonly encountered in drug discovery workflows, spanning DMSO-formulated small molecules and aqueous biologic reagents, which differ substantially in their physical properties and dispensing requirements.

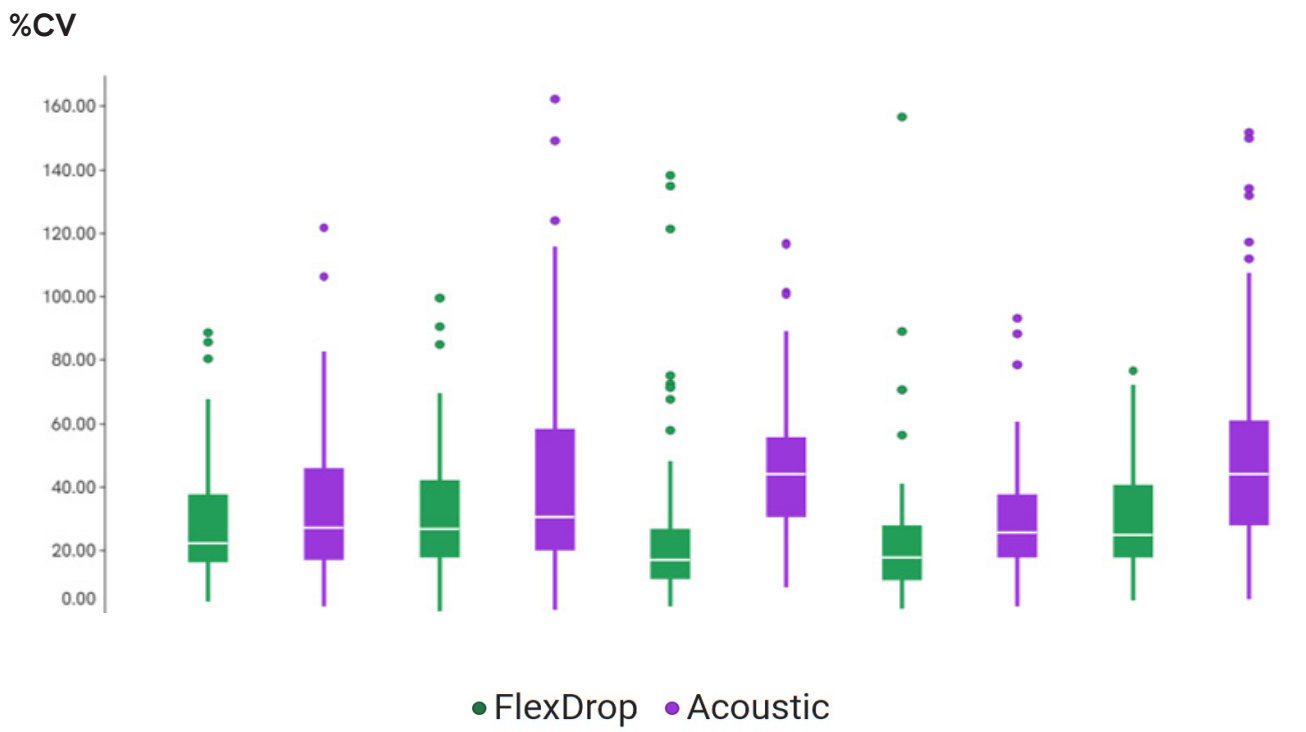


	SM X SM		mAB x SM		SM X ADC		ADC X mAb	
	FlexDrop	Acoustic	FlexDrop	Acoustic	FlexDrop	Acoustic	FlexDrop	Acoustic
Median	0.52	0.44	0.5	0.17	0.35	0.07	0.59	0.22

Figure 1: Z-factor analysis of assay robustness across four treatment classes in 3D spheroid assays. Z-factor distributions obtained using the FlexDrop dispenser (green) and acoustic dispensing (purple) are shown for small molecule × small molecule (SM × SM), monoclonal antibody × small molecule (mAb × SM), small molecule × ADC (SM × ADC), and ADC × monoclonal antibody (ADC × mAb) treatment classes. Data represents multiple screening experiments performed across five cell lines.

The FlexDrop dispenser consistently generated robust Z-factors across all treatment classes tested. For SM × SM combinations, both the FlexDrop dispenser and acoustic dispensing produced acceptable assay performance. However, differences between the dispensing platforms became more apparent when ADCs and monoclonal antibodies were incorporated. Under these conditions, the FlexDrop dispenser consistently delivered robust assay performance, while acoustic dispensing exhibited greater variability and broader Z-factor distributions, with the largest differences observed for ADC- and biologic-containing treatment classes.

Assay reproducibility was further assessed by calculating percent coefficient of variation (%CV) across multiple plates for each cell line and dispensing platform (Figure 2). Across all five cell lines evaluated, the FlexDrop dispenser consistently generated lower median %CV values and narrower data distributions than acoustic dispensing. Acoustic dispensing exhibited broader interquartile ranges and a greater frequency of outliers, while the FlexDrop dispenser maintained lower overall variability and more consistent performance across all cell lines tested.



	HCT-116		HCT-15		LoVo		SW1417		SW948	
	FlexDrop	Acoustic	FlexDrop	Acoustic	FlexDrop	Acoustic	FlexDrop	Acoustic	FlexDrop	Acoustic
Median	22.35	27.44	26.78	30.77	17.27	44.16	17.84	25.74	24.87	44.03

Figure 2: Assay reproducibility across five 3D spheroid cell lines. Distributions of percent coefficient of variation (%CV) values obtained using the FlexDrop dispenser (green) and acoustic dispensing (purple) are shown across spheroid of five cell lines.

The %CV analysis was consistent with the Z-factor results, with both metrics indicating improved assay robustness and reproducibility with the FlexDrop dispenser. Differences between dispensing platforms were most evident in treatment conditions containing ADCs and monoclonal antibodies, where precise and consistent low-volume delivery is critical for reliable assay performance.

Notably, a Z-factor of 0.5 is commonly regarded as a benchmark for excellent assay quality and is most often associated with highly optimized biochemical assays (Zhang et al., 1999). The FlexDrop dispenser achieved median Z-factors of 0.5 or greater for three of the four treatment classes evaluated, demonstrating robust performance despite the inherent biological variability associated with complex 3D spheroid assays.

Preservation of 3D spheroid integrity

To assess the impact of dispensing on spheroid morphology, A549 spheroids were imaged 96 h post-dosing following treatment with vehicle controls (DMSO and PBS) (Figure 3). A549 was selected as a representative 3D spheroid model for this analysis.

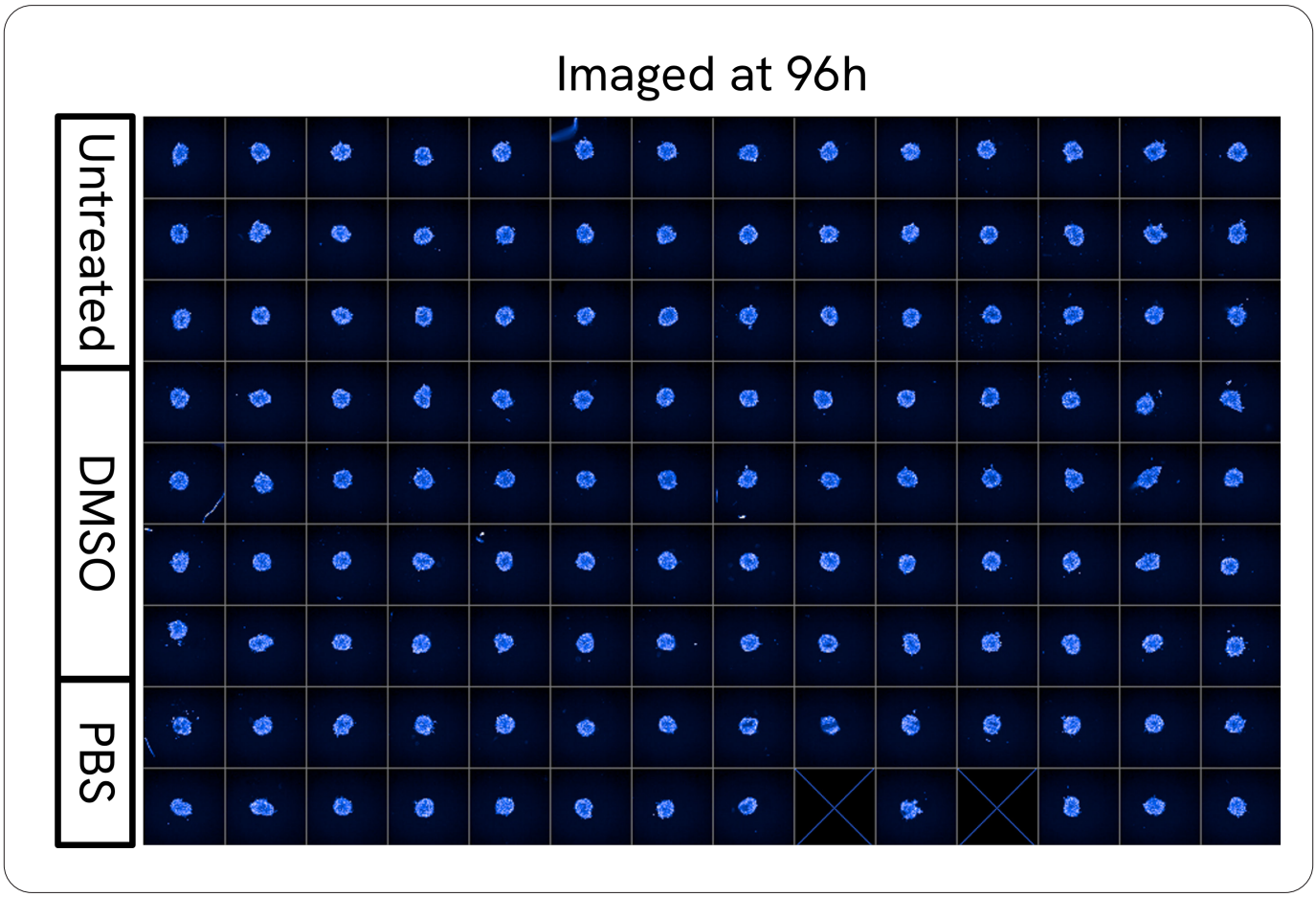


Figure 3: Assessment of 3D spheroid morphology following FlexDrop dispensing. Representative images of A549 spheroids acquired 96 h post-dosing following treatment with vehicle controls (DMSO or PBS) using the FlexDrop Plus Non-Contact Dispenser. Untreated, DMSO-treated, and PBS-treated spheroids are shown.

Across all treatment groups, including untreated, DMSO-treated, and PBS-treated wells, spheroids retained a compact, rounded morphology with no visible evidence of fragmentation or structural disruption. Vehicle-treated spheroids were morphologically comparable to untreated controls, with no observable changes in spheroid size, shape, or overall integrity. These observations indicate that neither the dispensing process nor vehicle addition adversely affected spheroid morphology over the course of the assay.

Pharmacological profiling in 3D spheroid assays

To determine whether improved dispensing performance translated into enhanced pharmacological data quality, the responses of the small-molecule payload SN-38 and the antibody–drug conjugate Labetuzumab Govitecan (LG) were evaluated in SW948 3D spheroid cultures (Figure 4). Single-agent dose–response curves extracted from the combination screening dataset are shown in Figure 4.

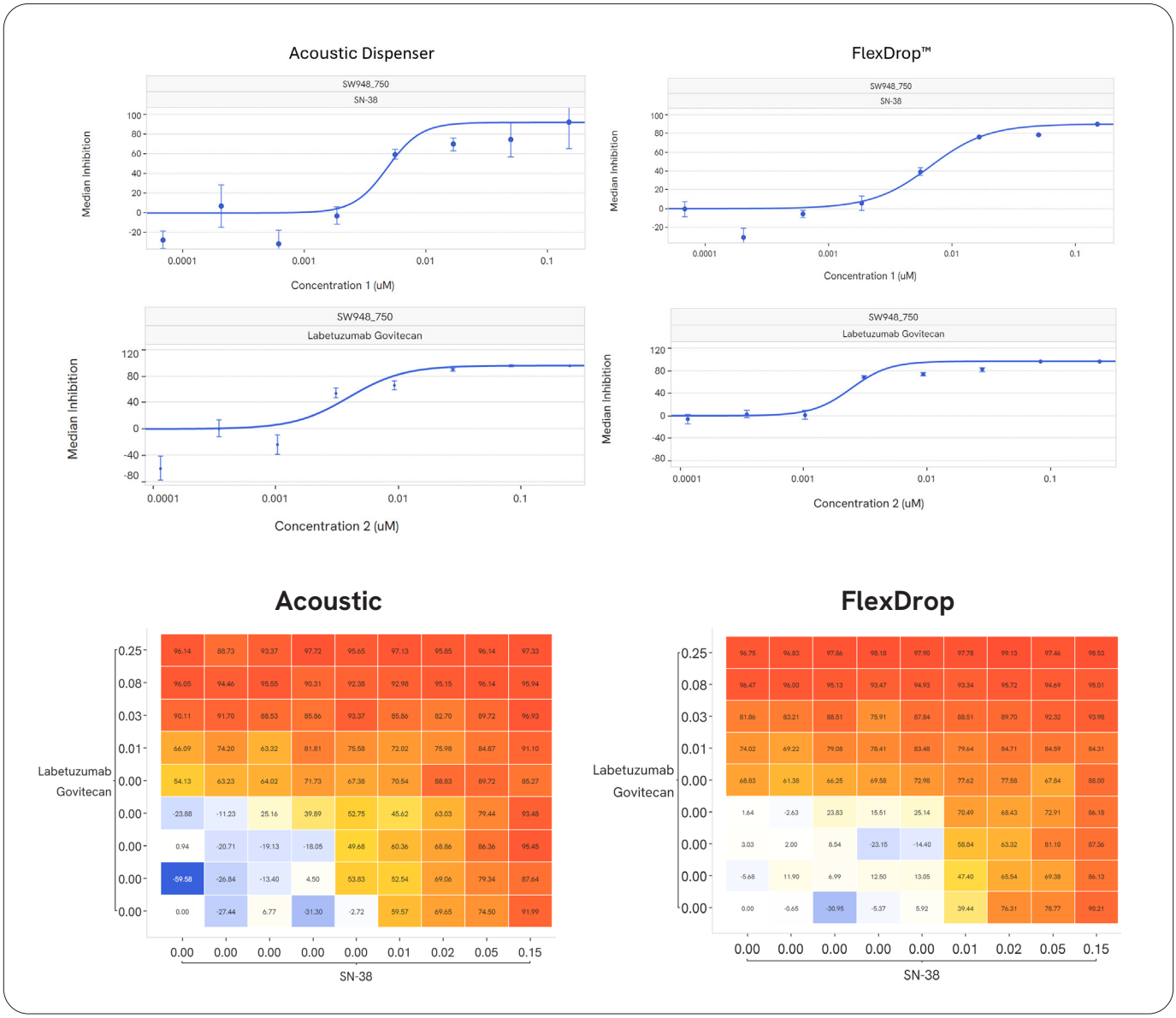


Figure 4: Pharmacological profiling of SN-38 and Labetuzumab Govitecan (LG) in SW948 3D spheroid cultures. Single-agent dose–response curves and combination inhibition matrices were generated using concentration-matrix experiments. Cell viability was assessed 96 h post-dosing using the ATPlite 1 glow-F luminescence assay. Data generated using the FlexDrop dispenser (left) and acoustic dispensing (right) are shown.

For SN-38, both dispensing platforms generated the expected concentration-dependent inhibition profile, with comparable curve shapes, potency, and maximal responses. However, the FlexDrop dispenser produced tighter replicate agreement across the concentration range, resulting in reduced variability and a more clearly defined dose–response relationship.

Differences between dispensing platforms were more pronounced for LG. While both methods generated comparable concentration-dependent responses, acoustic dispensing exhibited greater variability between replicates across the dose range. In contrast, the FlexDrop dispenser produced a more tightly defined dose–response profile with improved replicate agreement, particularly around the transition region of the curve. These observations are consistent with the assay robustness and reproducibility metrics described above and further highlight the benefits of consistent low-volume dispensing for ADC-containing assays.

Analysis of the complete SN-38/LG combination dataset demonstrated comparable pharmacological responses across both dispensing platforms, supporting consistent interpretation of the interaction between the two agents (Figure 4). Comparable inhibition heat maps were obtained using both dispensing approaches, indicating that the underlying biological conclusions were maintained across platforms.

Conclusion

The FlexDrop Plus Non-Contact Dispenser delivered robust and reproducible performance across a range of 3D spheroid screening applications, including assays incorporating ADCs, monoclonal antibodies, and small molecules. Improvements in assay quality relative to acoustic performance were demonstrated through increased Z-factors and reduced assay variability across multiple cell lines and treatment classes, with the greatest benefits observed in ADC- and biologic-containing assays.

Importantly, the FlexDrop non-contact dispensing did not adversely affect spheroid morphology, with vehicle-treated spheroids maintaining comparable size, shape, and structural integrity to untreated controls throughout the assay. Improved dispensing consistency also translated into tighter single-agent dose–response profiles, particularly for the ADC Labetuzumab Govitecan, supporting more reproducible pharmacological measurements.

While comparable pharmacological interactions were observed across both dispensing platforms, the FlexDrop dispenser consistently delivered enhanced assay robustness and reproducibility. Together, these results demonstrate the suitability of the FlexDrop dispenser for advanced 3D spheroid screening workflows, either run in house or by our preclinical services team in Cambridge and highlight its value for applications involving ADCs and other complex biologic molecules. As biologics and advanced 3D assay models continue to gain importance in drug discovery, reliable liquid handling will play a critical role in generating high-quality screening data, increasing confidence in experimental outcomes, and supporting more informed progression decisions.

References

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