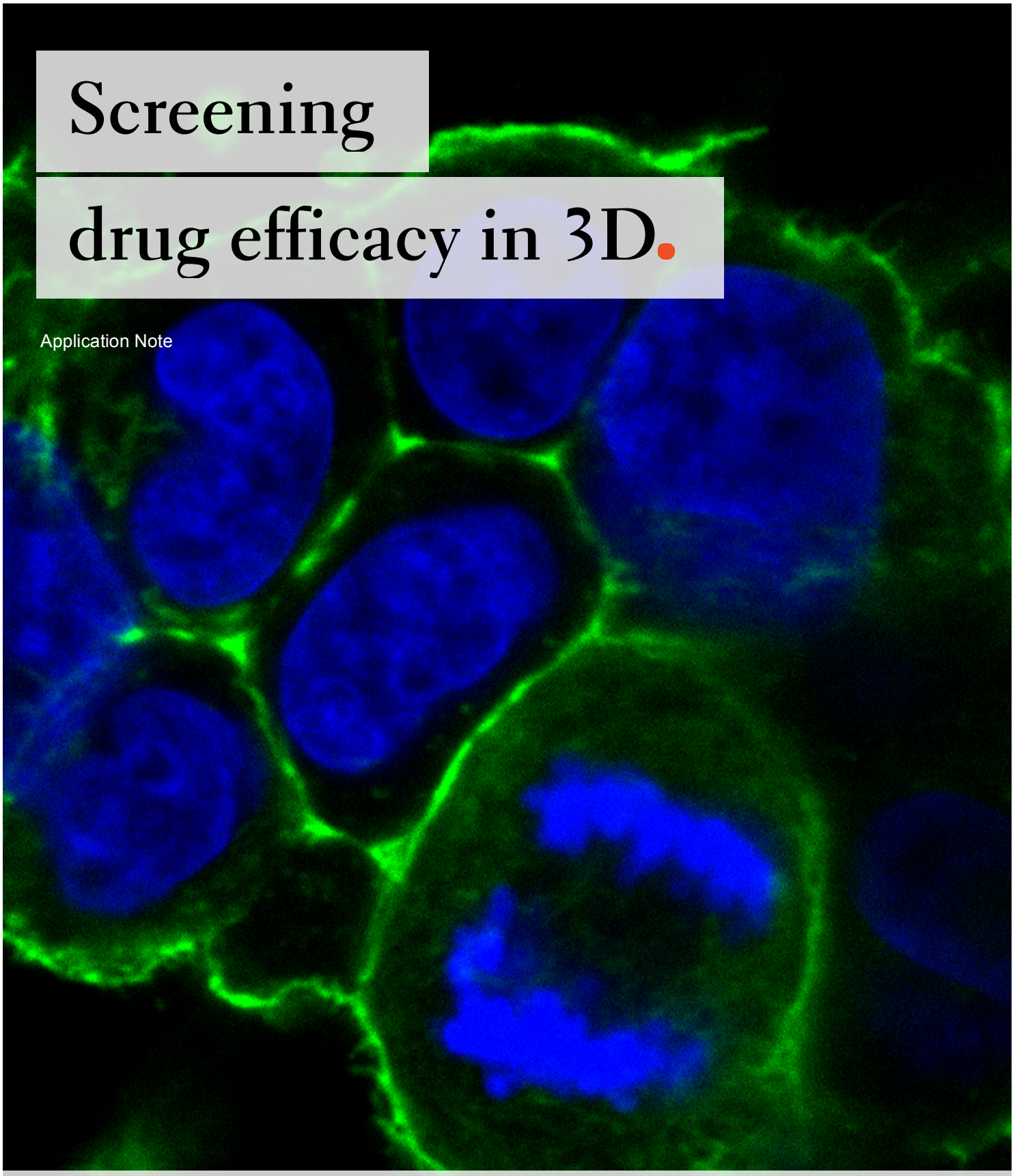
Screening drug efficacy in 3D.

Application Note



USING AUTOMATION TO OPTIMIZE 3D BLOOM MICROTISSUE CONDITIONS AND DRUG EFFICACY SCREENING IN AN OVARIAN CANCER CELL MODEL

TECAN.

INTRODUCTION

Cellular behavior is influenced by a broad variety of factors in vivo; atmospheric pressure, chemical signals, mechanical interactions with neighboring cells, nutrition supply and gas exchange, to mention just a few. For over thirty years, researchers have strived to mirror these complex factors in comprehensible model systems, and it is widely acknowledged that cells grown in a three dimensional (3D) environment mimic the natural conditions of a living organism much more closely than those grown in a flat 2D structure. Numerous studies have shown that the morphology, proliferation, metabolism and expression profiles of cells grown in 3D cultures demonstrate increased long-term viability and offer more reliable prediction of in vivo response to a potential drug. However, despite a multitude of technical advancements, 3D cell culture is considered a relatively young and complicated field of research, and 2D cell culture formats are still commonly used in drug discovery and screening leading to unsustainably high failure rates of new pharmaceuticals in preclinical testing.

CellSpring offers a new, easy-to-use technology – 3D Bloom® – that can generate complex 3D cell cultures for virtually any cell type. 3D Bloom uses a cell-friendly cross-linking reaction between two naturally-derived biopolymers to assemble cells into 3D structures in less than an hour. The system is not only highly biomimetic, it is also automation friendly and scalable, enabling medium to semi-high throughput, making it well suited to preclinical drug testing.

This application note describes the optimization of cell culture conditions for A2780 ovarian cancer cells in 3D Bloom on a Freedom EVO® liquid handling platform, as well as a fully automated drug efficacy screening protocol.

MATERIAL AND METHODS

Automated liquid handling

A Freedom EVO liquid handling platform was used to automate the preparation of 3D Bloom cultures in 96-well plates, set-up serial dilutions of the compounds, add and remove dosing media across the cell plate, and perform a CellTiter-Glo® Luminescent Cell Viability Assay (Promega, USA), as described in a previous application note. The platform was equipped with an eight-channel Air LiHa™, a Robotic Manipulator Arm™ (RoMa) and a Te-Shake™ heater-shaker module. A dust cover and sterile disposable tips were used to provide a semi-sterile environment, and carriers were placed on the workdeck to provide storage for reagent troughs, microplates and disposable tip boxes.

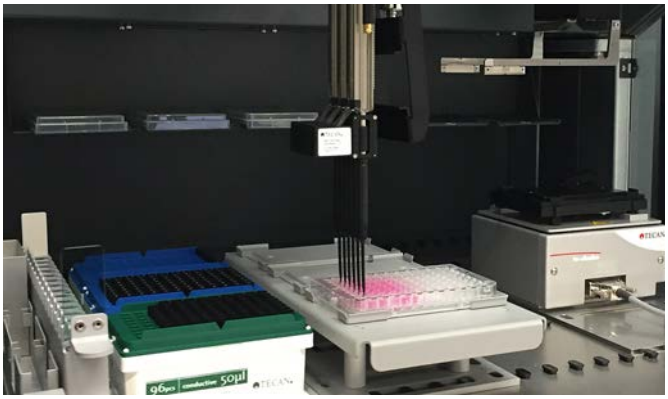


Figure 1: Deck layout of CellSpring's Freedom EVO workstation.

Optimization of 3D Bloom cultures for ovarian cancer cell line A2780

Culturing conditions for A2780 cells growing in 3D Bloom must be optimized to ensure the most effective model is used to test drug candidates. As a preliminary experiment, a CellTiter-Glo (CTG) assay was performed to evaluate the growth of A2780 cells in 3D Bloom gels. Cultures were prepared as previously described, using two different seeding densities (5,000 and 10,000 cells/well) and two different concentrations of 3D Bloom Solution A (7.5 and 10 mg/ml). Cell proliferation was assessed using the CTG assay, which provides a luminescence readout that is proportional to the cellular ATP content. Proliferation was measured at three time points (days 1, 4 and 7) with an offline Infinite® 200 PRO microplate reader, and bright field images of the 3D Bloom gels were taken at the same time points using an Invitrogen™ EVOS™ FL Auto Cell Imaging System (Life Technologies).

Drug efficacy screening

Dose-response curves and bright field imaging
A2780 cells were seeded (10,000 cells/well) in 3D Bloom and incubated for four days in standard growth media containing RPMI with GlutaMAX (Gibco, USA) and 10 % FBS (Gibco, USA). On Day 4, samples were treated with test compounds over the range 16ng/ml-25 µg/ml, including a vehicle control (no drug) on each plate. Comparator media was replaced by standard growth media after 24 hours, and the samples were incubated for 48 hours. Cell viability was assessed using the CTG assay

on the final day (day 7) – and the values reported as a percentage of the vehicle control – as well as bright field images of each well.

Confocal imaging to exemplify cell-drug interactions
To prepare for confocal microscopy, 3D Bloom samples were cultured in standard growth media for four days and then treated with 5 µg/ml of test compound for 24 hours. Samples were then washed three times in PBS (Gibco, USA) at room temperature, and fixed in 4 % formalin for 15 minutes. Samples were washed a further three times in PBS and permeabilized in 0.5 % Triton™ X-100 (Sigma-Aldrich) for 15 minutes, then washed three times in PBS and stained with Alexa Fluor® 488 phalloidin (1:50) (Thermo Fisher, USA) and 5 µg/ml DAPI (Thermo Fisher, USA) for one hour in the dark at room temperature. After a final wash in PBS, samples were imaged with a Leica SP8 confocal laser scanning microscope.

RESULTS AND DISCUSSION

Optimization of 3D Bloom

Figure 2 shows luminescence versus time for two seeding densities – 5,000 and 10,000 cells per well – and two concentrations of 3D Bloom Solution A (7.5 mg/ml and 10 mg/ml) of A2780 cells. The ideal conditions were considered to be 10,000 cells/well at a concentration of 10 mg/ml 3D Bloom Solution A, as the cells exhibited logarithmic growth during the testing period between days 4 and 7.

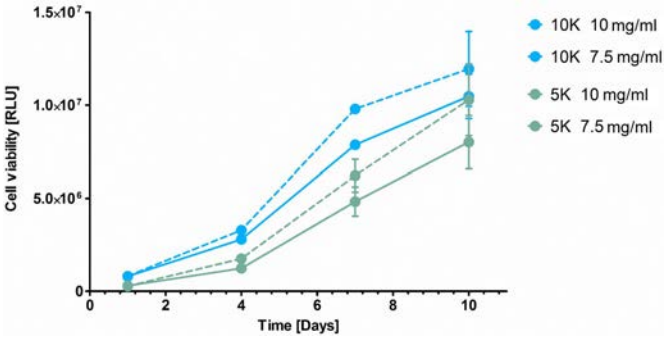


Figure 2: A2780 growth curves for optimization of conditions.

Bright field images were taken of each entire well under the optimized conditions, showing an increasing number of 'micro-tumor' clusters at days 4 and 7. Proliferation also increased over time, consistent with the corresponding growth curves. A2780 cells had the greatest proliferation in 3D Bloom microtissues seeded with 10,000 cells/well at 10mg/ml 3D Bloom Solution A, and so these conditions were chosen for the subsequent drug efficacy tests.

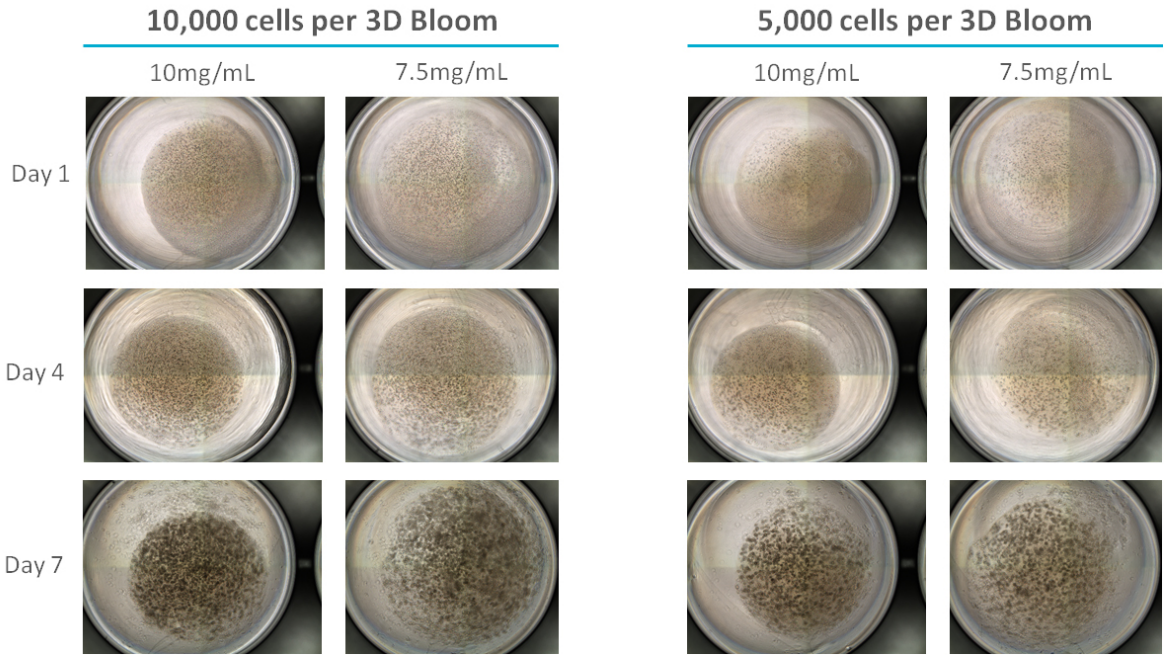


Figure 3: Bright field imaging of A2780 in 3D Bloom cultures comparing two cell seeding densities and two concentrations of 3D Bloom solution A.

Drug efficacy study

Uncontrolled cell division is a characteristic of tumors. In this study, three different compounds were tested in 3D Bloom, and the effect on cancer cell proliferation was measured to assess the potential of the drugs to specifically targeting dividing cancer cells.

Dose-response curves

Dose-response curves show the efficacy of a drug as the dosage is increased. More effective drugs show steeper curves, because cell viability decreases at a faster rate. Cell viability was assessed using the CTG assay, and plotted versus drug concentration (Figure 4). Cell viability decreased drastically as the dosage increased for both Drug A and Drug B, with both candidates providing a stronger dose-response than the marketed competitor.

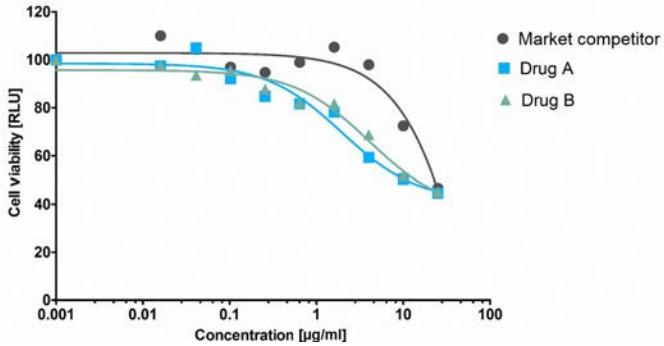


Figure 4: Dose-response curve for A2780 cells in 3D Bloom treated with test compounds.

Compound efficacy was ranked using GI_{50} values derived from the dose-response curves (Table 1). Compounds with a lower GI_{50} value require less drug in order to effectively inhibit cell growth. Drug A and Drug B have significantly lower GI_{50} values than the marketed competitor, demonstrating more effective inhibition of A2780 growth and resulting in a higher efficacy ranking.

Drug efficacy ranking	Compound	GI_{50} [µg/ml]
1	Drug A	1.95
2	Drug B	3.97
3	Market competitor	12.4

Table 1: GI_{50} values to rank compound efficacy.

Bright field imaging for assessment of drug efficacy

Bright field imaging at day 7 visually demonstrates the efficacy of the compounds. Compared to the untreated control, there is a clear difference in cell proliferation and viability for A2780s cultured in 3D Bloom cultures treated with 25 µg/ml of all three drugs. However, a significant number of viable cells remain when using the marketed competitor, whereas cells treated with Drug A or Drug B appear largely non-viable.

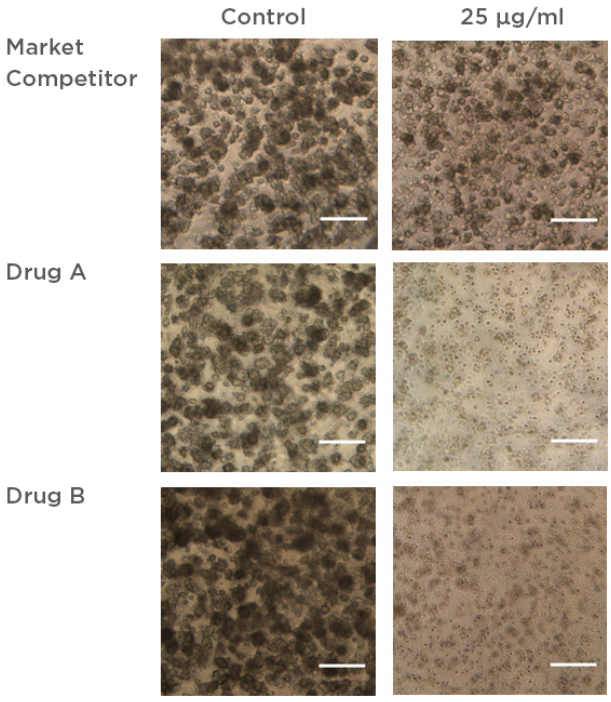


Figure 5: Bright field imaging to visualize compound efficacy.

Confocal imaging to exemplify cell-drug interactions.

Confocal fluorescence imaging shows the interaction of the drug (red) with the cell clusters (Figure 6). These images show that both Drug A and Drug B effectively penetrate the cell nuclei – shown by purple color – and Drug B also demonstrates some colocalization with cell membranes (yellow). In contrast, the marketed competitor appears to be less capable of penetrating the nuclei and shows little colocalization.

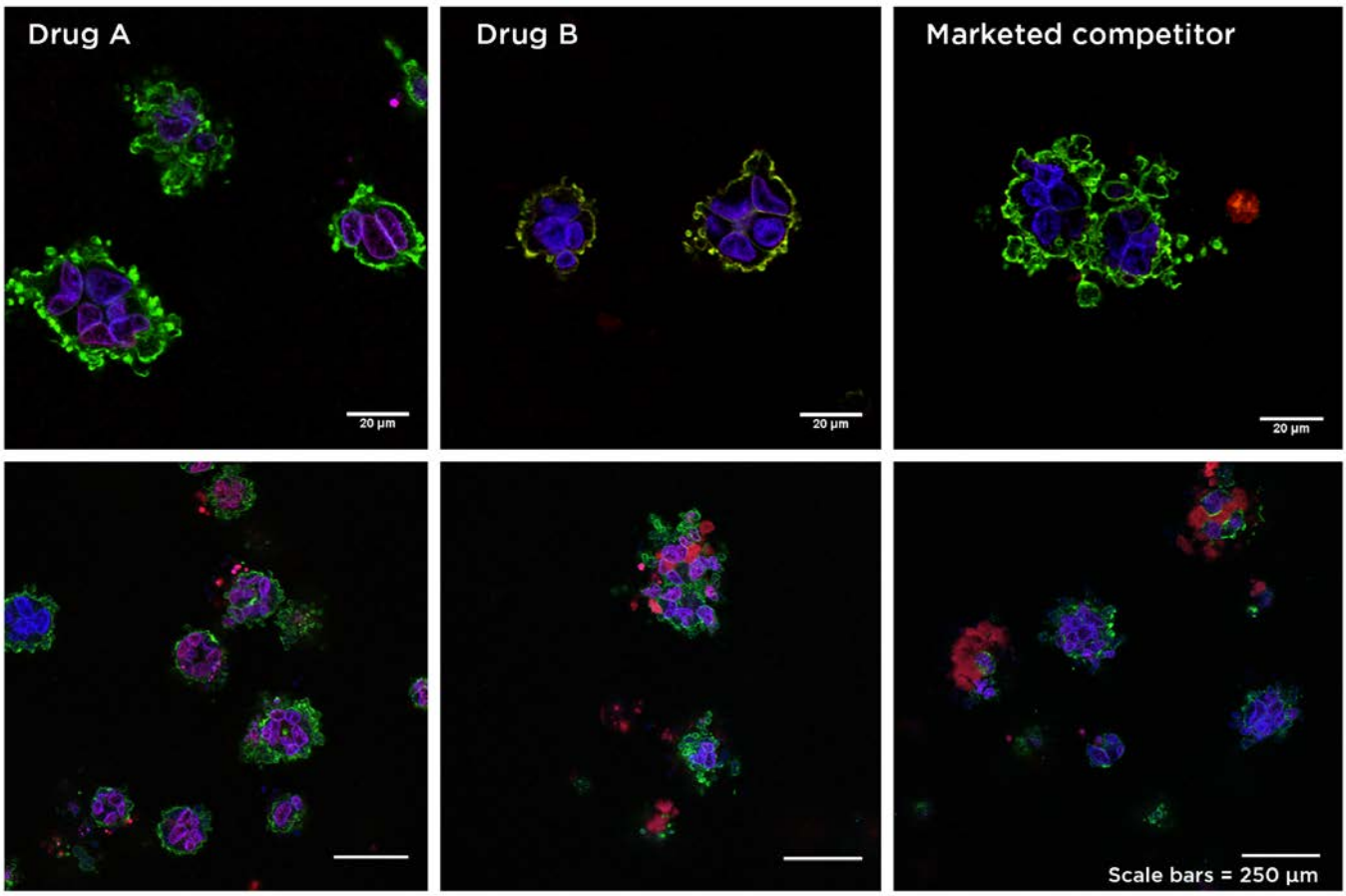


Figure 6: Immunostaining and confocal imaging to visualize drug-cell interactions.
Red = drug; Blue = DAPI (cell nuclei); Green = Alexa Fluor 488 phalloidin (actin cytoskeleton).

CONCLUSIONS

Fully automated preparation of 3D Bloom cultures can be performed in a reproducible and reliable manner on a Freedom EVO workstation. The platform can also be used for culture condition screening of the A2780 ovarian cancer cell line in a 96-well microplate format.

Furthermore, the 3D Bloom technology provides a versatile model to study the efficacy of candidate drugs using microscopy and a cell viability assay. Automation on the Freedom EVO offers the flexibility and reproducibility necessary to perform drug efficacy screens in 3D Bloom microtissues.

About the authors

Jocelyn Bailey studied bioengineering at the University of California, Los Angeles. Her previous research and work focused on tissue engineering, assay development, and in vitro diagnostics, and now she is continuing to develop the 3D Bloom technology alongside the Tecan platform.

Jana Langhoff holds a MSc in molecular and cell biology from the Free University of Berlin. Before joining Tecan as an application specialist for cell biology and protein science in 2015, her main focus lay on cancer research and studying neurodegenerative diseases.

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400276 V1.0 06-2017

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