Automated 3D Cell Culture Workflow Enabling Low Variation and Extended Hands-Off Time Along a Complete Tumor Treatment Assay

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Introduction

The development of automated 3D cell culture workflows is crucial for advancing research in drug discovery by enabling efficient and reproducible processes. This study explores the automation of Akura™ Spheroid Microplates using an epMotion® automated liquid handling system to establish a robust 3D cell culture process.

Our project focused on developing and validating automated methods for spheroid formation, drug treatment, and immunostaining using Akura™ 96 and 384-well microplates. HCT116, a human colorectal carcinoma cell line, was utilized due to its ability to form compact spheroids with a well-defined border mimicking 3D tumor structures. Drug treatment experiments were conducted using a clinically-relevant chemotherapy treatment combining folinic acid, 5-fluorouracil, and oxaliplatin (FOLFOX).

Results of the study include successful spheroid formation with consistent size and viability, comparable IC_{50} values between manual and automated processes, and encouraging results towards automated immunostaining. Overall, these experiments show a high repeatability in 96 and 384-well plates with a coefficient of variability <10%.

Workflow automation using the epMotion® and Akura™ Microplates offers a promising solution for medium-throughput 3D cell culture applications. The outlined automated approach enhances reproducibility and hands-off time, while reducing manual labor and human errors, making it a valuable tool for researchers to enhance efficiency and precision in their work.

Technology and methods

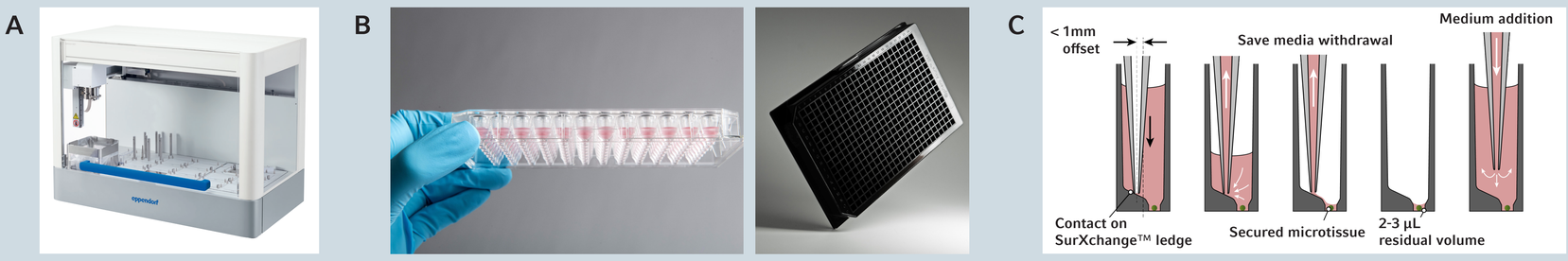
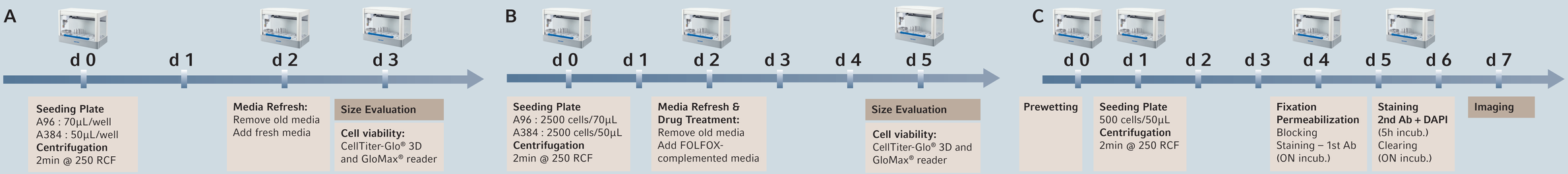


Figure 1 (left): The epMotion® 5075t (A) is an automated liquid handling system that offers precision and flexibility for scientific workflows. Akura™ 96 and 384-well Spheroid Microplates (B) are designed for reliable spheroid formation, cultivation, and analysis. Special well bottoms allow for simple and efficient media exchanges (C).

Figure 2 (bottom): Workflows for spheroid culture (A), drug treatment (B), and immunostaining (C).



Automated culture using Akura™ Spheroid Microplate technology

Results demonstrate that 3D cell culture automated on the epMotion using Akura™ Microplates is feasible and effective. Fig. 3 indicates that size and cell viability of spheroids are consistent across manual and automated processes, as well as in 96 and 384-well plate formats.

Repeatability was assessed by measuring the coefficient of variation (CV%). The automated process provides reproducible results in larger batches with a CV% below 10%, making it a valuable tool for increasing throughput from 96 to 384 samples in 3D cell culture applications.

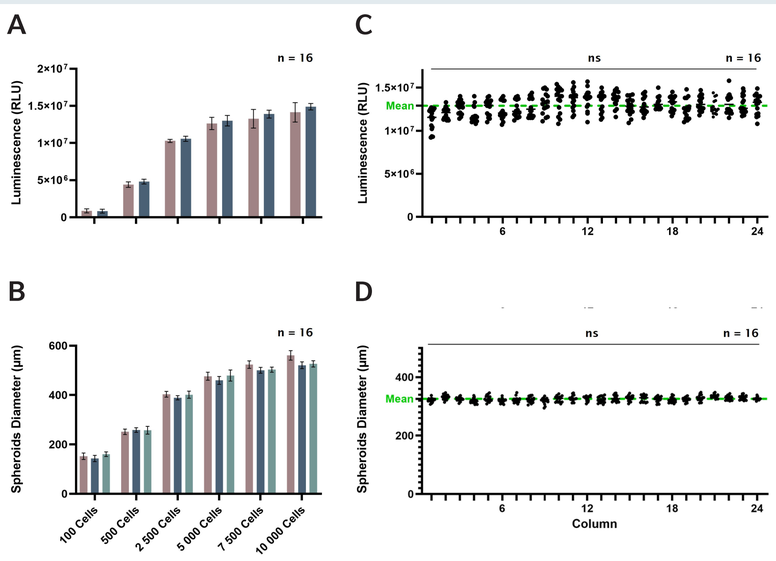


Figure 3: Results of automated spheroid culture. Comparison of cell viability (A) and spheroid size comparison (B) by manual and automated processing, as well as homogeneity in automated processing in terms of cell viability (C) and spheroid size (D). Cell viability was measured 3 days after seeding and assessed using CellTiter Glo® 3D (Promega, G9682). Spheroid Sizes were measured using ImageJ software. Homogeneity of cell viability and spheroid size was assessed column-wise at 2,500 cells in 384-well format. Significance was calculated by comparison of individual columns to the total mean. Key: red: manual processing 96-well, blue: automated processing 96-well, green: automated processing 384-well. ns: not significant.

Automated immunofluorescent staining of spheroids using the epMotion®

Spheroid staining in Akura™ 384 Microplates was demonstrated through an immuno-fluorescent staining procedure automated via epMotion (Fig. 7). Seven days are necessary to complete the entire procedure in 384-well format, including more than 35 h of automated runtime. Images show a good homogeneity of the staining for different cell inputs (Fig. 6). Evaluation of individual wells show good repeatability with a homogeneous cell coloration throughout the plate (not shown).

While the initial results of the immunostaining procedure are promising, further optimization is required to minimize the risk of spheroid loss. In addition to increased reproducibility and minimization of human error, the epMotion provides notable hands-off time (35h) in a very long and complex procedure (Fig. 8).

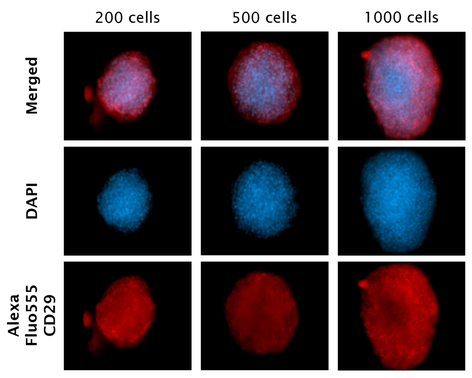


Figure 6 : Stained spheroids at different input cell numbers per 50 µL. Images show blue coloration of nuclei (DAPI) and red coloration of CD29-beta integrin (Alexa Fluo555). Confocal images acquired by ZEISS Celldiscoverer 7.

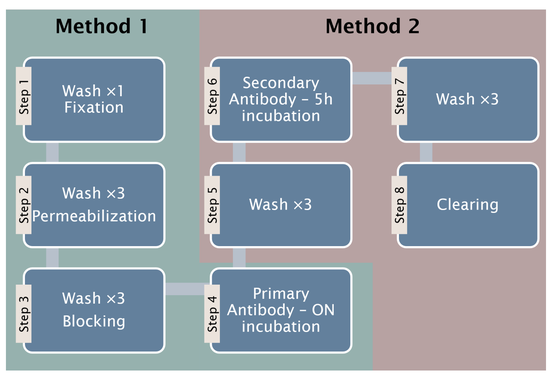


Figure 7 : epMotion® methods to run the spheroid immunostaining procedure. Incubations from step 4 and 6 are achieved using the Akura™ plate lid. ON: overnight.

Medium throughput 3D spheroid culture and drug screening

Images of FOLFOX-treated spheroids show the impact of drug concentration on cell growth and cell viability (Fig. 4). As anticipated, a higher concentration of drugs leads to a significant decrease of cell viability and spheroid size in comparison to untreated cells. Moreover, increasing concentrations are impacting the previously well-defined border of spheroids.
The results obtained in this study demonstrate that the IC_{50}-value is between 3.5 and 5 µM of FOLFOX, regardless of the process or the plate format (Fig. 5). The automation of drug screening in 384-well format is reducing error-prone treatment and limit exposition to hazardous reagents for the researcher during the process.

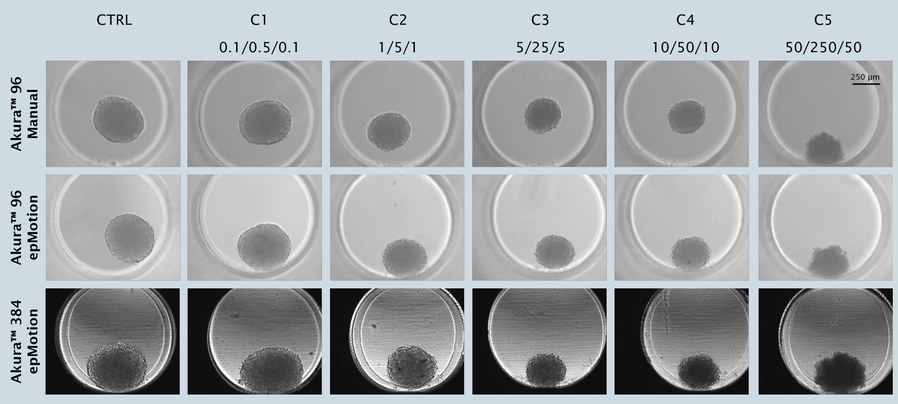


Figure 4: Images of spheroids two days after FOLFOX treatment. 2,500 cells were treated with five different drug concentrations from 0.1 to 50 µM. Acquisition was done using EVOS FL Auto2 (Invitrogen). Well diameter: 1mm. Numbers below C1–5 labels indicate concentrations [µM] of folinic acid, 5 fluorouracil, and oxaliplatin, respectively.

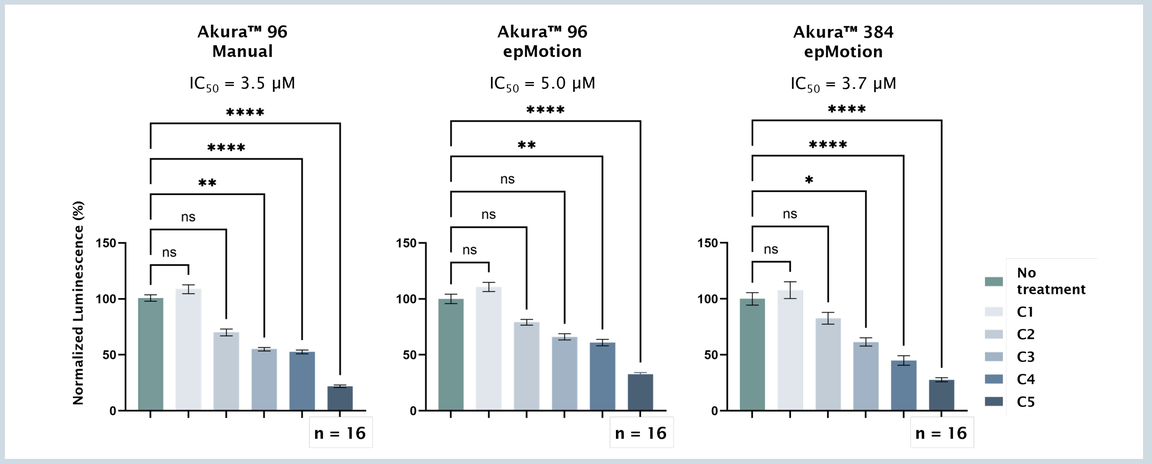


Figure 5: Cell viability measured for five different FOLFOX concentrations obtained from manual and automated processing in 96 and 384-well format. Luminescence values were normalized (RLU) in relation to untreated spheroids. Statistics have been calculated by non-parametric Kruskal-Wallis test. Concentration values as depicted in Fig. 4. ns: not significant.

Conclusions

The integration of epMotion with Akura™ Spheroid Microplates provides an efficient solution for 3D cell culture and drug screening, enabling researchers to enhance the reproducibility and throughput of experimental work-flows in cellular biology and drug discovery.

In collaboration with InSphero

www.eppendorf.com/epMotion