Tech Note

Media Exchanges Using Unattached 3D Spheroid Models

A Quantitative Comparison between Manual and AMX™ Automated Media Exchange Based Methods

Introduction

3D cell culture methods encourage cell-cell and cell-matrix interactions and also promote more physiological cell morphologies and behaviors compared to cells grown on flat 2D surfaces. Due to the need to generate the most *in-vivo* like data during *in vitro* drug discovery of toxicology testing, 3D spheroids are often used in long-term experimental procedures, and in microplate format to increase throughput. Since these procedures may extend to weeks, media exchange and dosing steps are critically important. When incorporating unattached spheroids, care must be taken not to evacuate or damage the spheroid suspended in the culture media.

Here, we compare microplate-based 3D spheroid media exchange methods using an electronic handheld multichannel pipette with vacuum force, and the patent-pending AMX™ Automated Media Exchange module on the MultiFlo™ FX Multi-Mode Dispenser. The AMX module consists of two unique, modified peristaltic pump cassettes with eight stainless steel tube aspirate and dispense heads. Cassette tubing is fed through the MultiFlo FX peristaltic pumps and into media bottles or tubes. Software controls the pumps to run slowly and gently so as to not disturb the spheroids during aspirate or dispense cycles. Each cassette is fully autoclavable, enabling sterile processing.

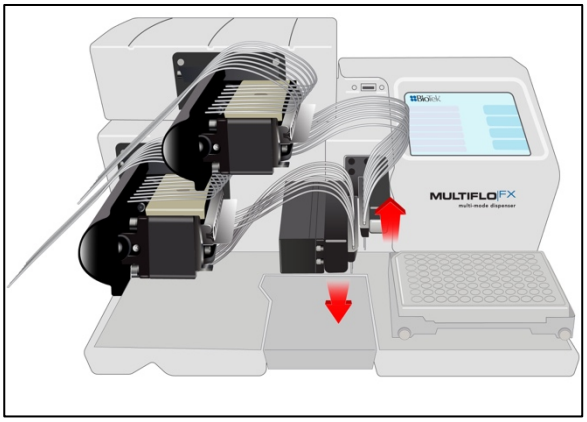

Figure 1. MultiFlo FX Multi-Mode Dispenser equipped with the AMX Automated Media Exchange module, showing aspirate (right arrow) and dispense (left arrow) heads.

Spheroid Formation

HCT116 colorectal carcinoma cells in media at a concentration of 2000 cells/well were manually dispensed into odd numbered columns of a 96-well round bottom microplate; and the same concentration was automatically dispensed into evenly numbered columns of the same microplate using the MultiFlo FX and its AMX module. Total volume in each well was 100 µL. The plate was then incubated for 48 hours to allow the cells to self-aggregate into spheroids.

Manual and Automated Media Exchanges

After spheroid formation, the plate was returned to the MultiFlo FX with AMX module, where the aspirate tubes were positioned at the back, right corner of each evenly numbered column well, and 85 µL of media was removed at a rate of 15 µL/second. The same volume was slowly aspirated from each odd numbered column well using the electronic handheld multichannel pipette.

Fresh media was then added to the plate. Using MultiFlo FX with the AMX module, dispense tubes were positioned at the back, right corner of each evenly numbered column well, and 85 µL of media was added at an optimized dispense rate of 20 µL/second. The same volume of media was slowly manually dispensed into each odd numbered column well. The manual and automated media exchange process was repeated twice to simulate a typical fluorescence assay or antibody staining wash protocol.

Image-Based Comparisons

Following the first and third manual and automated media exchange cycle, brightfield imaging was performed, using the Cytation™ 5 Cell Imaging Multi-Mode Reader to analyze the status of spheroids in wells.

A.

B.

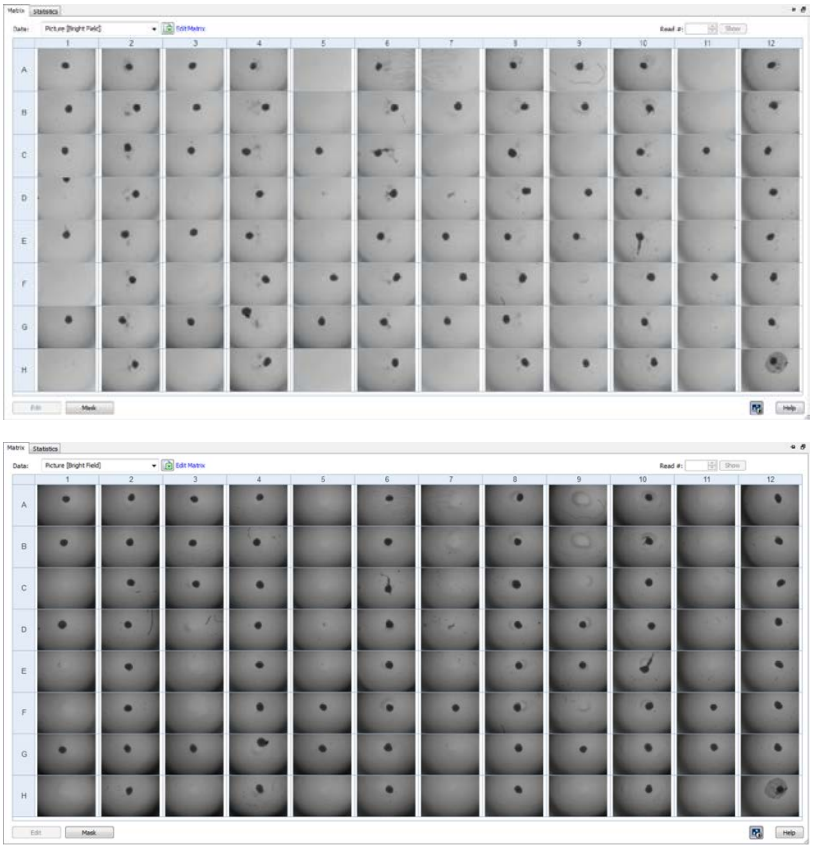

*Figure 2. Brightfield images of the entire 96-well microplate after (**A.**) one; and (**B.**) three media exchange cycles. Media exchanges were performed manually in all odd columns, and automated using the MultiFlo FX equipped with the AMX Automated Media Exchange module in all even columns.*

As seen in Figure 2, a total of 23 spheroids (48%) were evacuated after one media exchange, and 32 spheroids (67%) were evacuated after three media exchanges using the manual method, while no spheroids were removed using the MultiFlo FX equipped with the AMX Automated Media Exchange module. Note that in Figure 2 (**A.**), there is not a visible spheroid in well G11, yet in 2 (**B.**), a spheroid is clearly visible in that same well. With manual methods, spheroids may not be removed, but can be moved within the well such that it is not imaged, rendering that replicate unusable.