Automated workflow for 3D cell model dispensing using spheroONE and phenotypic profiling using Pu·MA System EC

Anthony Eritano[2], Ekaterina Nikolov[1], Andreea Merloiu[1], Alissa Schutte[2], Alyssa Rogers[2], John Olthoff[2], Gerri Ortiz[2], **Evan F Cromwell[1]**
[1]PFI, Inc. 875 Cowan Rd, Suite B, Burlingame CA 94010, [2]Scienion, Inc. 1231 W. Warner Rd, Suite 103, Tempe AZ 85284

INTRODUCTION

Three-dimensional cell models have gained popularity, compared with traditional 2D models, because they better reproduce key aspects of human tissues and are amenable to a wide range of applications from basic research to pharmaceutical drug safety and efficacy testing. Methods for generating 3D models, such as organoids, spheroids, and tumoroids, have progressed to where scientists have the ability to recapitulate most human organs and cancer types ex vivo.[1,2] However, the transition from 2D to 3D cell models has resulted in challenges related to sample handling and assay development and more sophisticated protocols and instrumentation are required.[3] Here we present a novel automated workflow for phenotypic profiling of 3D models using spheroONE® for automated sorting, isolation, and dispensing of spheroids, and Pu·MA System® EC with low attachment flowchips for performing viability assays.

The spheroONE is a large-particle sorter and dispenser that uses precision dispensing technology together with advanced image-based sorting capabilities to select and isolate single spheroids. The system was optimized to dispense size-selected spheroids into the protected sample chamber of Pu·MA System flowchips.[4,5] The Pu·MA System EC with temperature, CO_2 and relative humidity control was used to incubate the spheroids in the flowchips and perform viability assays using automated fluid transfers. The samples were stained in the flowchips with viability dyes and then imaged and analyzed. We used this workflow to analyze proliferation and viability of HCT116 colon cancer spheroids and found spheroONE results consistent with manual dispense methods. This automated workflow using spheroONE and Pu·MA System EC for 3D cell model dispensing and phenotypic profiling eliminates sample disturbance, manual handling errors and provides consistent reproducible high-quality data. This platform is a valuable tool in a wide range of research areas including disease modeling, drug discovery and personalized medicine.

INSTRUMENTATION

The Pu·MA System EC and 3D Flowchip features:

- Automated media exchanges occur with cells in protected chamber
- Supernatants can be collected to monitor cell secretion
- Spheroids can be imaged in the flowchip, or samples removed for immunoassay or metabolomics analysis
- Assay protocols can be edited via the Pu·MA System Software

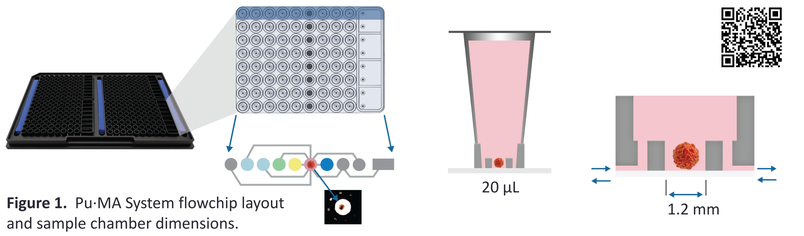


Figure 1. Pu·MA System flowchip layout and sample chamber dimensions.

spheroONE features:

- Up to 100% accurate automated isolation of single cellular aggregates using morphology (size and shape)-based sorting.
- Can sort and isolate cell aggregates between 50 and 600 µm in diameter.
- Gentle dispensing technology maintains the integrity and viability of fragile cellular aggregates.
- Improved cellular debris removal compared to manual single aggregate isolation.
- Compatible with standard multi-well plates (e.g., 94 and 384) in addition to custom designed labware.
- Direct Visual inspection of the sample along with full image record of isolated cell aggregates.

References
1. Drug resistance profiling if a new triple negative breast cancer patient-derived xenograft model. Matossian, M.D. et al (2019) BMC Cancer. 19(1): 205
2. In-depth characterization of a new patient-derived xenograft model for metaplastic breast carcinoma to identify viable biologic targets and patterns of matrix evolution within rare tumor types. Matossian, M.D. et al (2021) Clin. & Trans. Oncology 24: 127-144
3. High-Content Assays for Characterizing the Viability and Morphology of 3D Cancer Spheroid Cultures. Sirenko, O. et al (2015) 13, 402, ADDT
4. Disease modeling with 3D cell-based assays using a novel flowchip system and high-content imaging. Cromwell, E.F. et al (2021) SLAS Tech. 26(3): 237
5. Multifunctional profiling of triple-negative breast cancer patient-derived tumoroids for disease modeling. Cromwell, E.F. et al (2022) SLAS Disc. 27: 191.
6. Didox and resveratrol sensitize colorectal cancer cells to doxorubicin via activating apoptosis and ameliorating P-glycoprotein activity. Khaleel, S.A. et al (2016) Sci Reports 6, 36855

SPHEROID ISOLATION AND DISPENSE

Precise isolation and dispensing of 3D cell models such as spheroids and organoids is critical for assay automation. It is important that downstream assay steps are done without loss or damage to the organoids. Consumables need to be compatible with automated imaging systems and other assay readout modalities to provide high quality data.

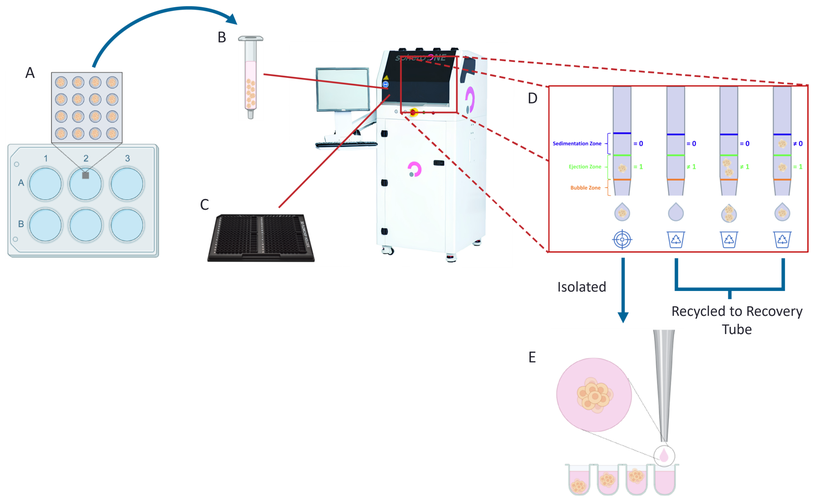


Figure 2. SpheroONE standard workflow to isolate spheroids. (A) Cells are grown and placed into spheroid forming microcavity plate. (B) When spheroids are formed, they are transferred to a sample reservoir. (C) Sample and desired MTP plate are loaded into the spheroONE®. (D) Using Image-Based Isolation Software, the spheroONE images a dispensing capillary. The capillary is divided into three zones: The bubble Zone (orange) represents the volume displaced during dispense. The Ejection Zone (green) represents volume of media that will be dispensed in next drop. The Sedimentation Zone represents the region where spheroids settle and can potentially be dispensed. (E) The software ensures that only when a single spheroid is in the Ejection Zone and no spheroids are in the Sedimentation Zone are isolated into individual wells.

SPHEROID ASSAY WORKFLOW

Assay reagents are loaded into wells of the flowchips and then fluid exchanges occur with the sample chamber according to pre-determined assay protocols. Spheroids were protected during these operations and approximately 95% of fluid is exchanged. This makes for very efficient wash and minimizes compound carry-over. The assay protocol here included compound incubation, viability staining, and wash steps. Spheroids were characterized by automated high resolution transmitted light and fluorescence imaging.

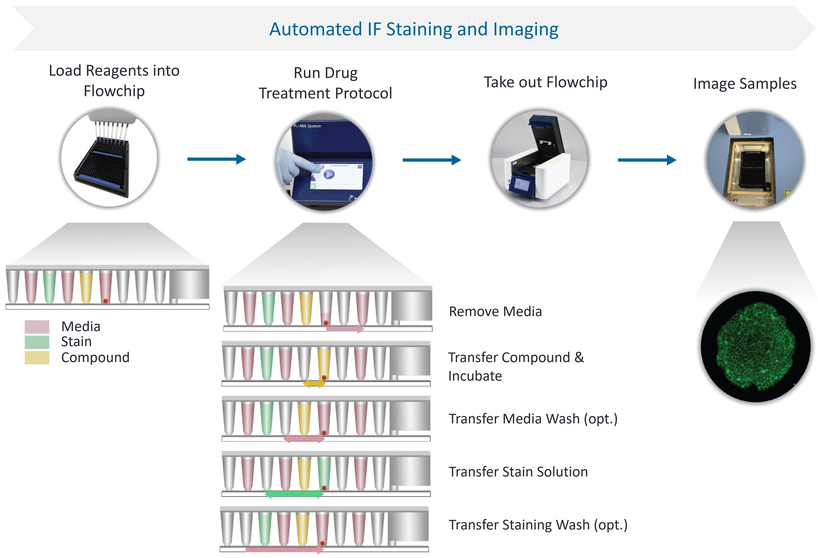
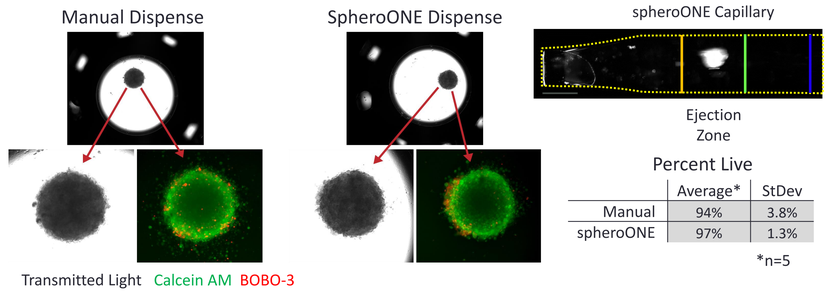


Figure 3. Workflow of spheroid viability studies. Spheroids are incubated in Pu·MA System flowchips, then media is exchanged with staining solution using Pu·MA System EC. Spheroids are imaged in the flowchips using automated high-content imaging systems (Yokogawa CQ1 Confocal & ECHO Revolution epifluorescence).

VIABILITY & COMPOUND RESPONSE

An important design criteria of the spheroONE is to not adversely affect spheroid viability. To characterize this sister spheroids were dispensed into flowchips using manual pipetting and spheroONE automated dispense. The sample wells were filled with 20 µL of media (McCoy's Complete) and incubated overnight. Media was replaced with viability staining solution using Pu·MA System and then spheroids were imaged for Live and Dead cells. Equivalent phenotypes and viability were observed between two methods with % Live cells > 90%.



Percent Live

	Average*	StDev
Manual	94%	3.8%
spheroONE	97%	1.3%

*n=5

We used this workflow to analyze proliferation and viability of HCT116 colon cancer spheroids. Several hundreds of HCT116 spheroids were formed in a microwell cavity plate (Aggrewell 800, Stemcell Technologies) and collected into 5 mL of media. They were then dispensed into the protected sample chamber of flowchip samples wells. Spheroids were treated for 72 hours with doxorubicin then stained with viability dyes using Pu·MA System. The spheroids were imaged in the flowchips using CQ1 confocal imaging system (Yokogawa) and analyzed for number of Live and Dead cells (see **Fig 4**). Spheroid viability results with IC_{50} of 1.6 µM were found to be consistent with published response of the compounds for 2D cell cultures (0.96 µM).[6] Spheroids were stained with calcein AM and BOBO-3, or CyQuant Green and EtHD-1, and imaged. Image stacks were analyzed for percent Live (calcein AM or CyQuant pos.) and Dead (BOBO-3 or EtHD-1 pos.) cells.[3]

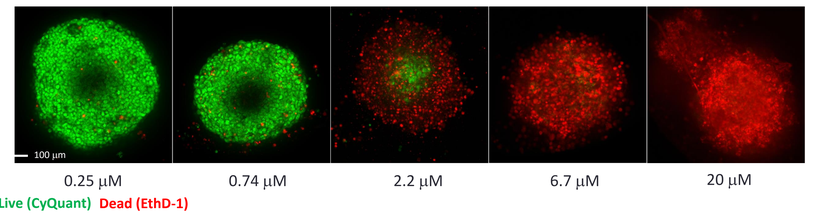
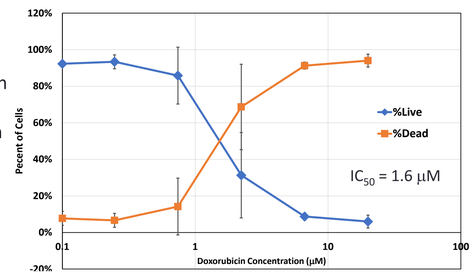


Figure 4. **Above:** Composite MIP images of HCT116 spheroids treated with different concentrations of doxorubicin for 72-hours. Spheroids were stained with CyQuant Green and EthD-1 (Invitrogen) for 2 hours. **Right:** Viability-response curve for incubation with compound. Thresholding was done independently in Live (Green) and Dead (Red) channels using a proprietary AI algorithm.. Percent response was determined from normalized integrated intensities. (Error bars = +/- 1 SD, n=3)



CONCLUSIONS

- We have demonstrated capabilities of a novel automated spheroid and organoid assay system that performs complex protocols with 3D cell models in an incubator environment.
- Spheroids and organoids are automatically sorted, isolated and dispensed into flowchips for downstream assays providing precise control over the size and number of spheroids.
- Assays and fluid exchanges are performed in a novel microfluidic device that protects the cell models and enhances precision and control of the assay steps.
- The ability to analyze spheroids and organoids *in situ* in order to capture toxicity information and perform functional assays shows great promise for disease modeling.

SCIENION A BICO COMPANY

PFI