

Automated assays with 3D tumor models using spheroONE and phenotypic profiling using Pu-MA System EC



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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, CO₂, 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.

AUTOMATED REAGENT EXCHANGE

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

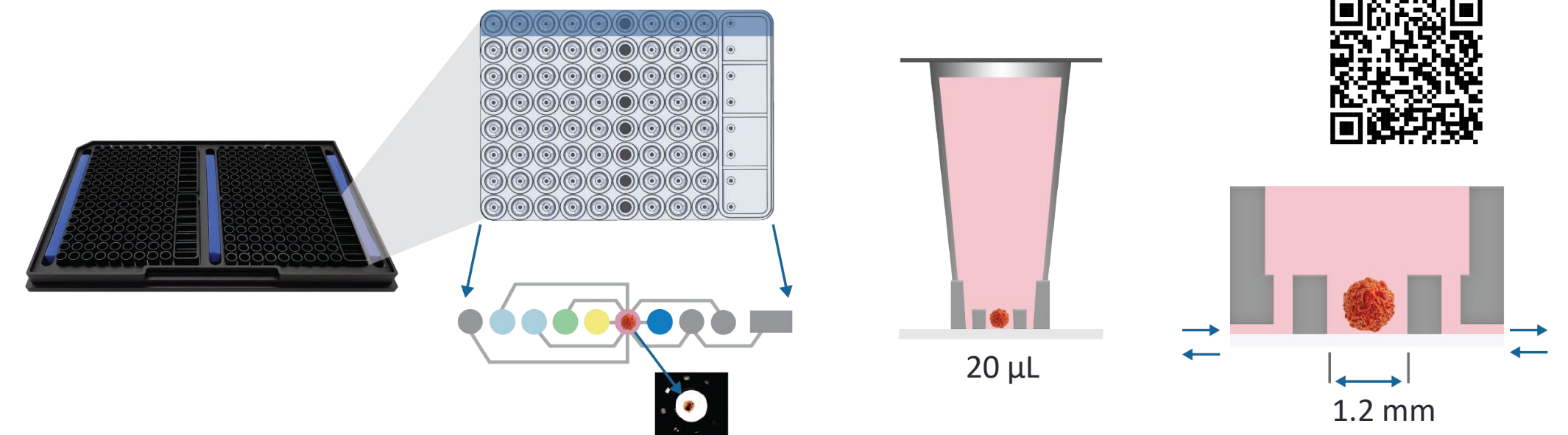
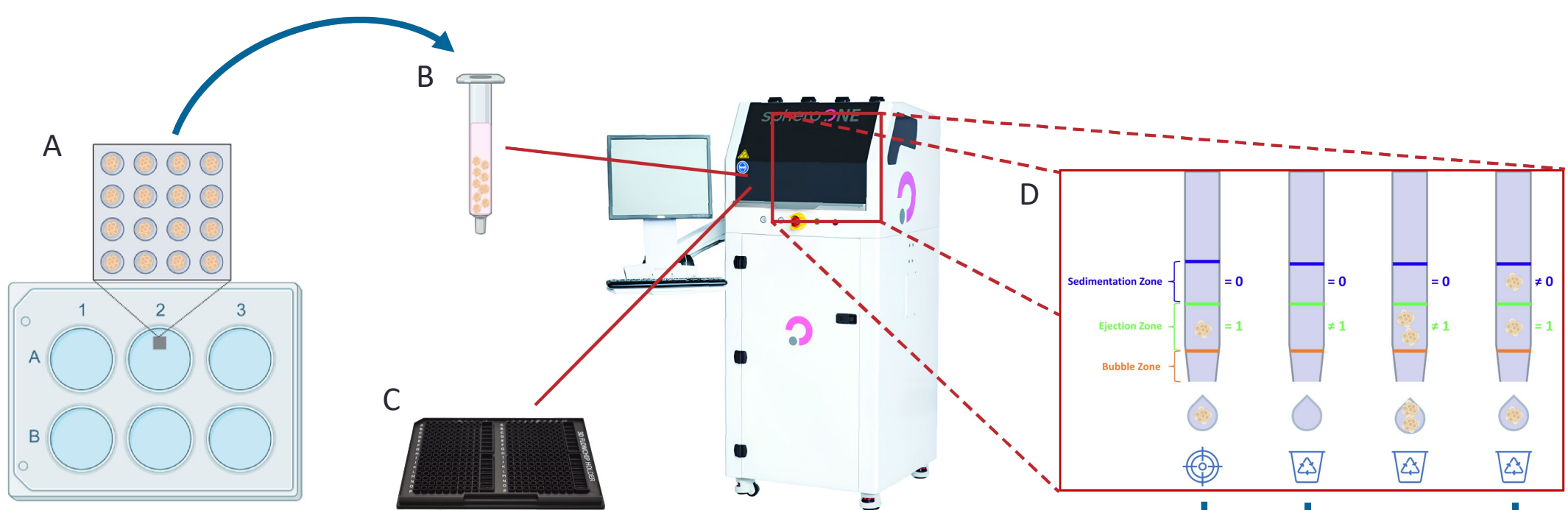


Figure 1. Pu-MA System flowchip layout and sample chamber dimensions.

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.



spheroONE features:

- Up to 100% accurate automated isolation of single cellular aggregates using morphology (size and shape) - based sorting. (50 to 600 μm dia.)
- Gentle dispensing technology maintains the integrity and viability of fragile cellular aggregates.
- Direct Visual inspection of the sample along with full image record of isolated cell aggregates.

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 flowchip wells 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.

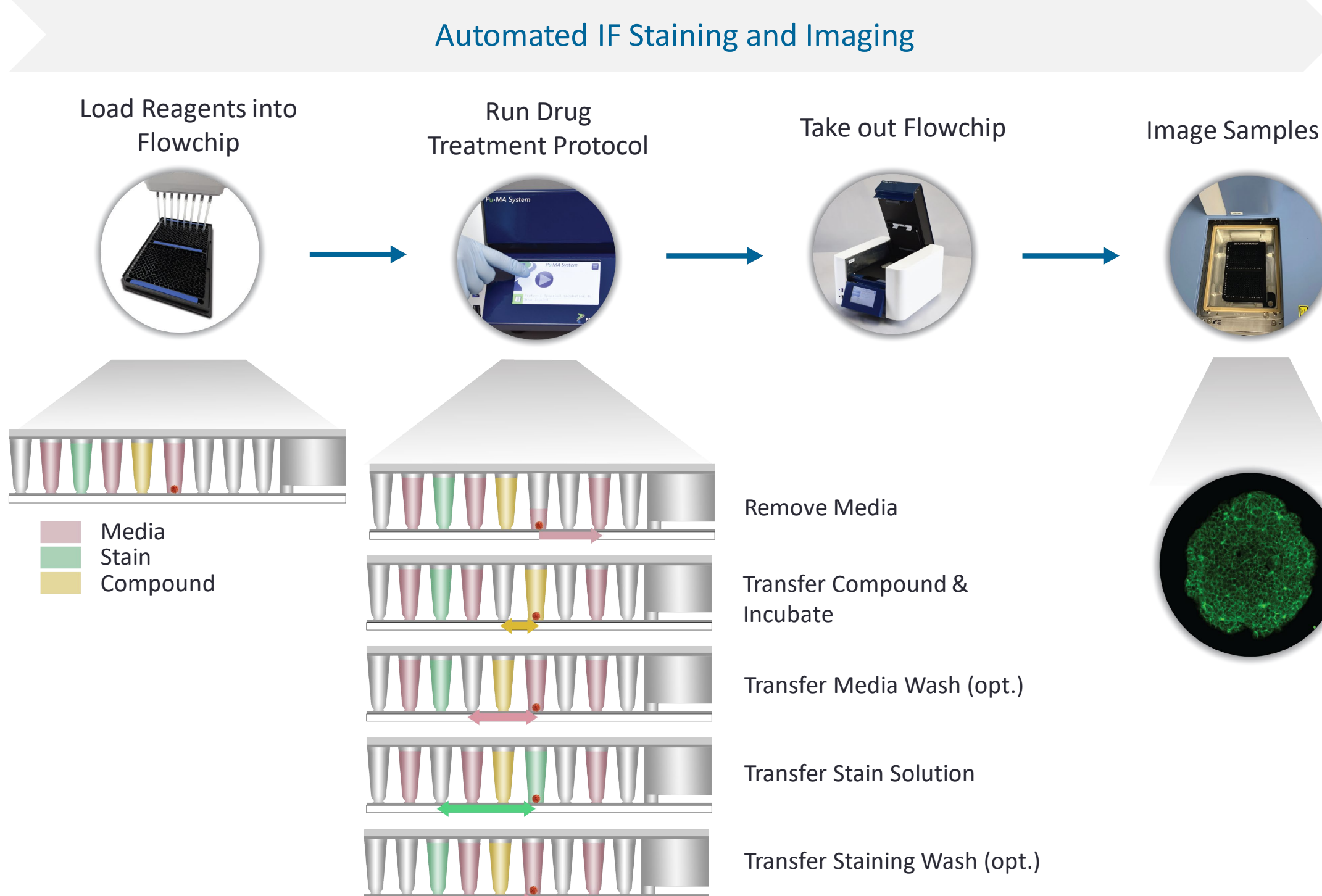


Figure 3. Workflow of spheroid viability studies. Spheroids are incubated in Pu-MA System flowchips, then media is exchanged with staining solution using Pu-MA System EC. Spheroids are imaged in the flowchips using automated high-content imaging systems (Yokogawa CQ1 Confocal & ECHO Revolution epifluorescence).

SPHEROID VIABILITY

An important design criteria of the spheroONE is to not adversely affect spheroid viability. To characterize this 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%.

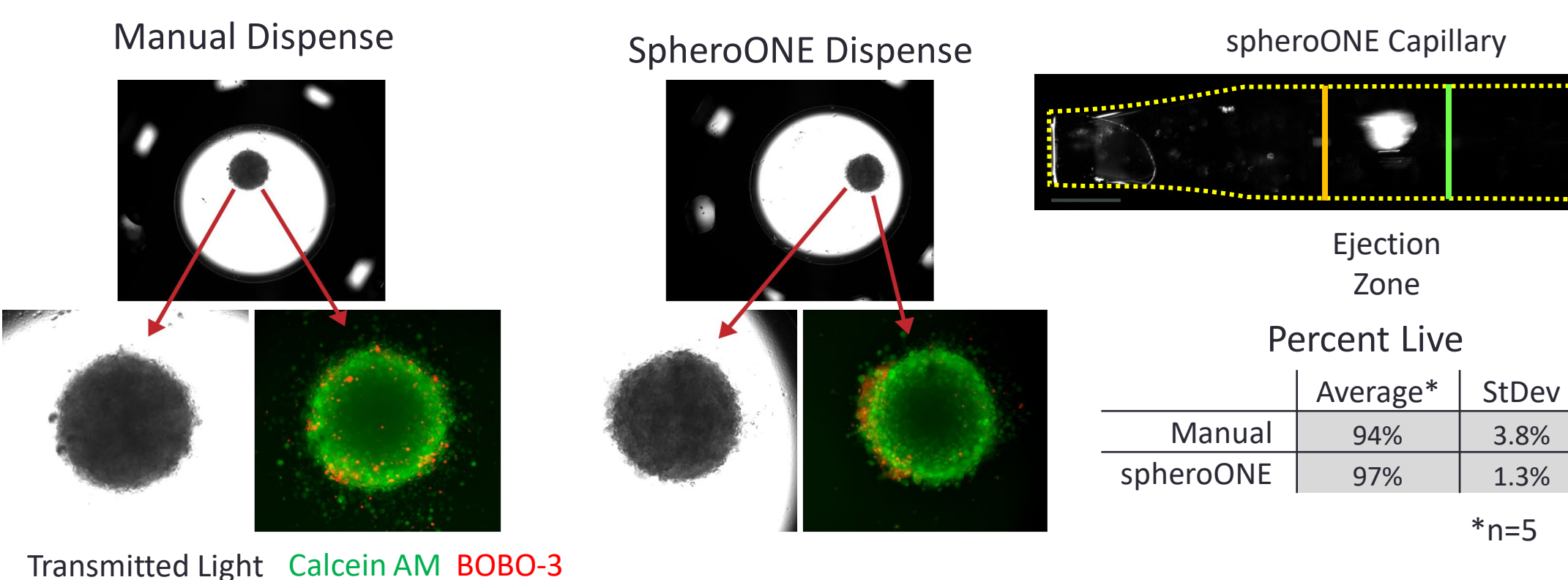
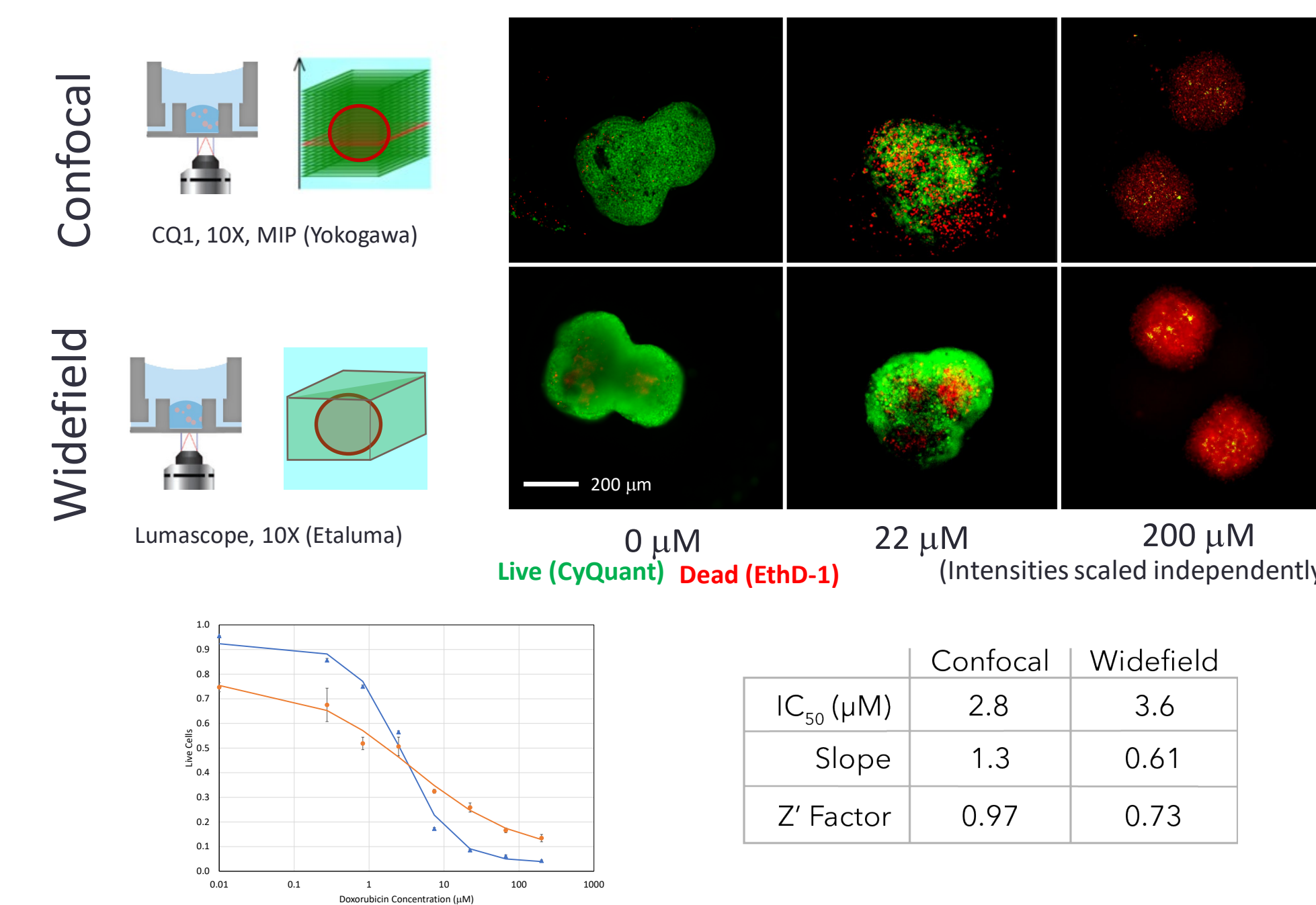


Figure 4. Comparison of spheroid viability using manual and spheroONE dispense in Pu-MA System flowchips.

WIDEFIELD VS CONFOCAL IMAGING

Fluorescence imaging is a key readout for viability assays. Confocal imaging is the gold standard for imaging 3D cell models; however, it has significant time overhead due to multiple Z-plane acquisition, produces large data sets, and requires complex image analysis. Widefield microscopy acquires data from all Z-planes at once which gives it advantages with speed and ease of use. We have developed AI-assisted analysis of tumoroid viability response and compared widefield vs confocal microscopy on the same spheroids.



References

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AI-ASSISTED VIABILITY ANALYSIS

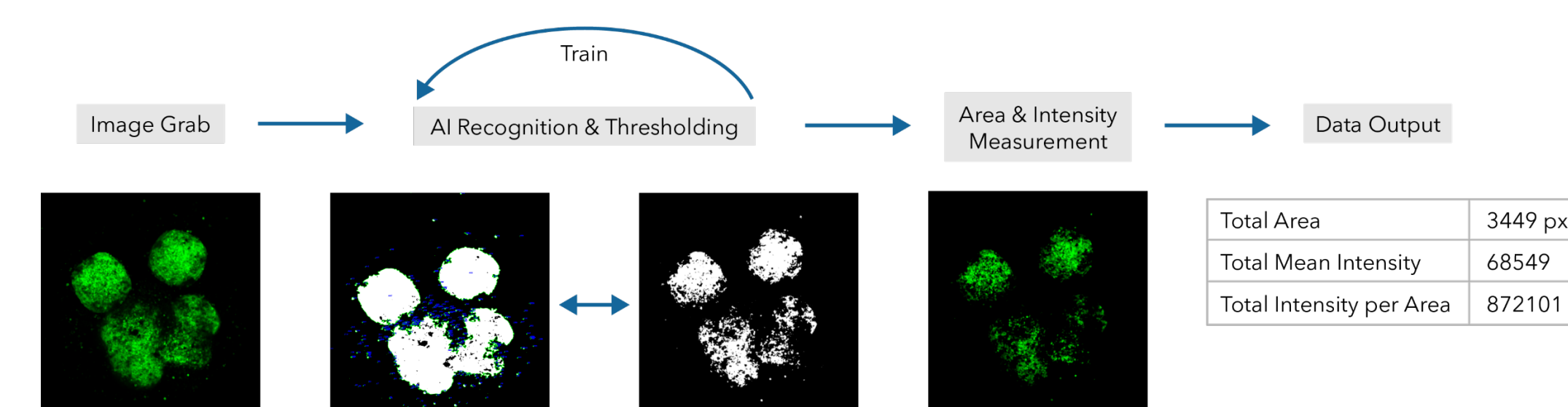


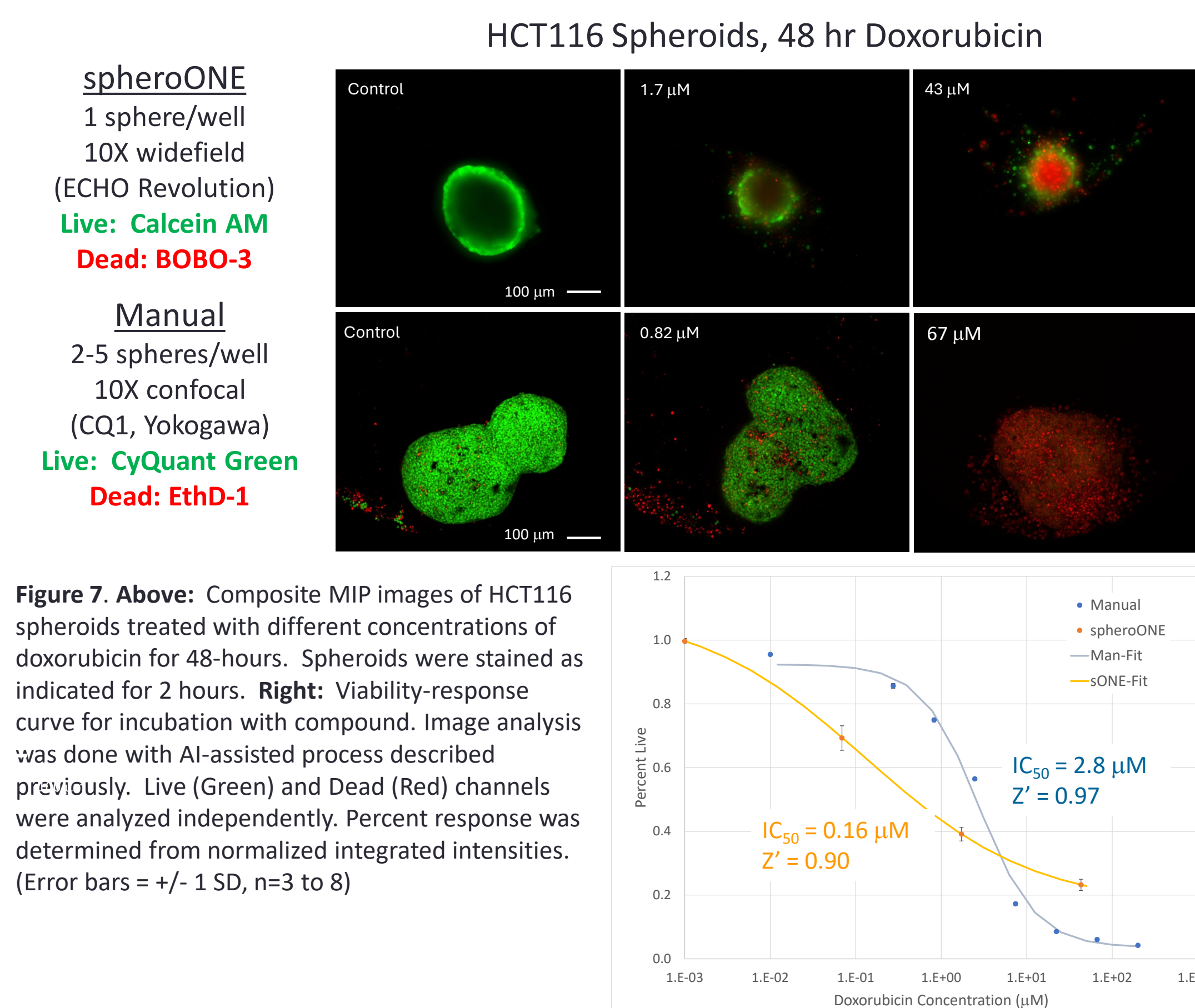
Figure 5. Workflow of AI-assisted analysis of fluorescently stained spheroids for viability assessment.

We have developed a comprehensive method for automated spheroid detection and analysis of fluorescent images, employing state-of-the-art deep learning techniques. The approach (see Fig 5) combines the power of transfer learning using a pre-trained VGG16 regional convolutional neural network (R-CNN) with custom-built fully connected layers for precise spheroid detection.

- Images undergo preprocessing operations to ensure compatibility with the VGG16 model (standardization of image size and intensity normalization).
- Pre-trained VGG16 R-CNN is employed to extract features from the images.
- Custom-built fully connected layers learn discriminative features through iterative training specific to spheroid detection.
- The model, facilitated by the Adam optimizer and binary cross-entropy loss function, measures spheroid parameters and produces data outputs.

COMPOUND RESPONSE

HCT116 spheroids were formed in a microwell cavity plates over 72 hr (Aggrewell 800, Stemcell Technologies, 2000 cells/cavity or Sphericalplate 5D 6-well, Heidolph, 600 cells/cavity) and collected into media. Spheroids were dispensed into flowchips sample wells using either spheroONE or manual pipetting. Spheroids were treated for 48 hours with doxorubicin then stained with viability dyes using Pu-MA System. The spheroids were imaged in the flowchips and analyzed for number of Live and Dead cells (see Fig 7). Spheroid viability results were found to be consistent with published response of the compounds for 2D cell cultures (0.96 μM).⁶



- The viability and phenotypic response to doxorubicin of spheroids dispensed via spheroONE was found to be comparable to manual dispense.
- Control of number of spheroids per well is more robust using spheroONE.
- The difference in slope of the concentration response is attributed to widefield versus confocal imaging and is consistent with previous results.
- The IC₅₀ was found to be an order of magnitude lower in the spheroONE experiment. This could be due to difference in doxorubicin preparation, spheroid formation methods, or other assay conditions. Further experiments are being undertaken to understand the shift.

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.