

Evaluation of T-cell Cytotoxicity, PD-L1 Expression and Phenotypic Features in Novel 3D Microfluidic Breast Cancer Co-culture Platform .

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INTRODUCTION

Immunotherapy is now recognized as a powerful therapeutic approach to treat and cure cancer. However, the objective response rates for most solid tumors are low (below 30%). Understanding the tumor - immune cell microenvironment provides insight into the complex interactions within the tumor between different cell populations and how to effectively target these interactions for treatment. With advances in immunotherapy there is a need for complex 3D tumor models because they more accurately represent the complexity and diversity of tumor microenvironments and cancer-immune cell interactions which influences the outcome of anti-cancer therapies. The increased complexities of 3D models add new challenges for 3D assay and advanced co-culture assay systems development.

We present an approach to perform **3D co-culture assays** using **automated microfluidic flowchips and Pu-MA System**. In this study breast cancer spheroids were co-cultured with T-cells from human PBMCs. The assay format presented combines moderately high level of biological complexity and a more predictable behavior with simpler reproducible protocols.

The platform allows the integration of:

- 3D cell model with delivery of immune cells + media exchanges
- Drug delivery
- Imaging capability to access phenotypic features
- Secretory activity profiling using biochemical-based readouts.

INSTRUMENTATION

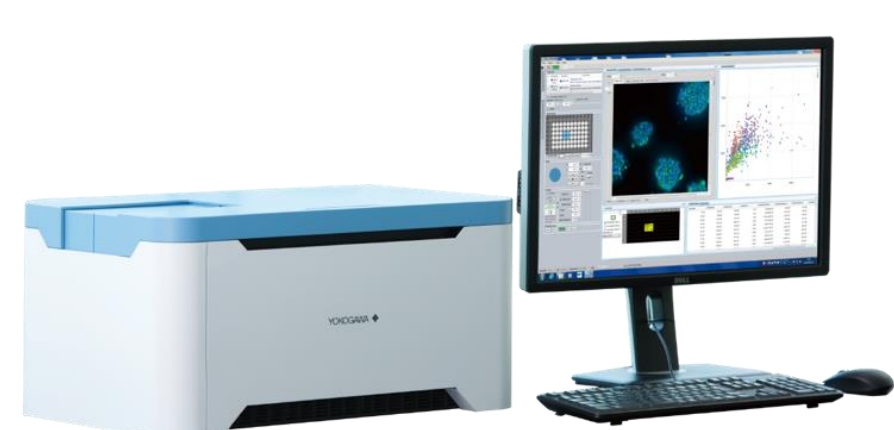
The Pu-MA System and flowchips

- Protected Sample Chamber technology prevents sample loss and damage
- Scheduled automated media exchanges to support 3D culture
- Scheduled automated collection of supernatants for secreted factors analysis
- Automated workflow solutions for IF Staining and other 3D cell assays
- In-Flowchip imaging



The CellVoyager CQ1 Benchtop High-Content Analysis System

- Confocal spinning disc technology
- High precision stage incubator and low phototoxicity
- Four fluorescence channels + transmitted light
- Integration with CellPathfinder high content analysis software



MICROFLUIDIC FLOWCHIP

Each Pu-MA System flowchip contains eight lanes of reagent wells connected by microfluidic channels. Four flowchips are placed in holder that locates all wells in a 384 multiwell plate format providing for 32 samples per assay. Each test lane is designated to one organoids/spheroid sample and consists of a sample well connected to 8 reagent wells. Flowchip wells can be filled with any reagents (media, compounds, stains, etc.) depending on the assay configuration. Organoids/spheroids are loaded into the sample well and located in a protected chamber at the bottom of the well. This allows reagents to be directed in and out of the sample well without disturbing or drying out the microtissue. The bottom of the flowchip is a thin cyclic olefin copolymer (COC) film which makes it compatible with high resolution imaging.

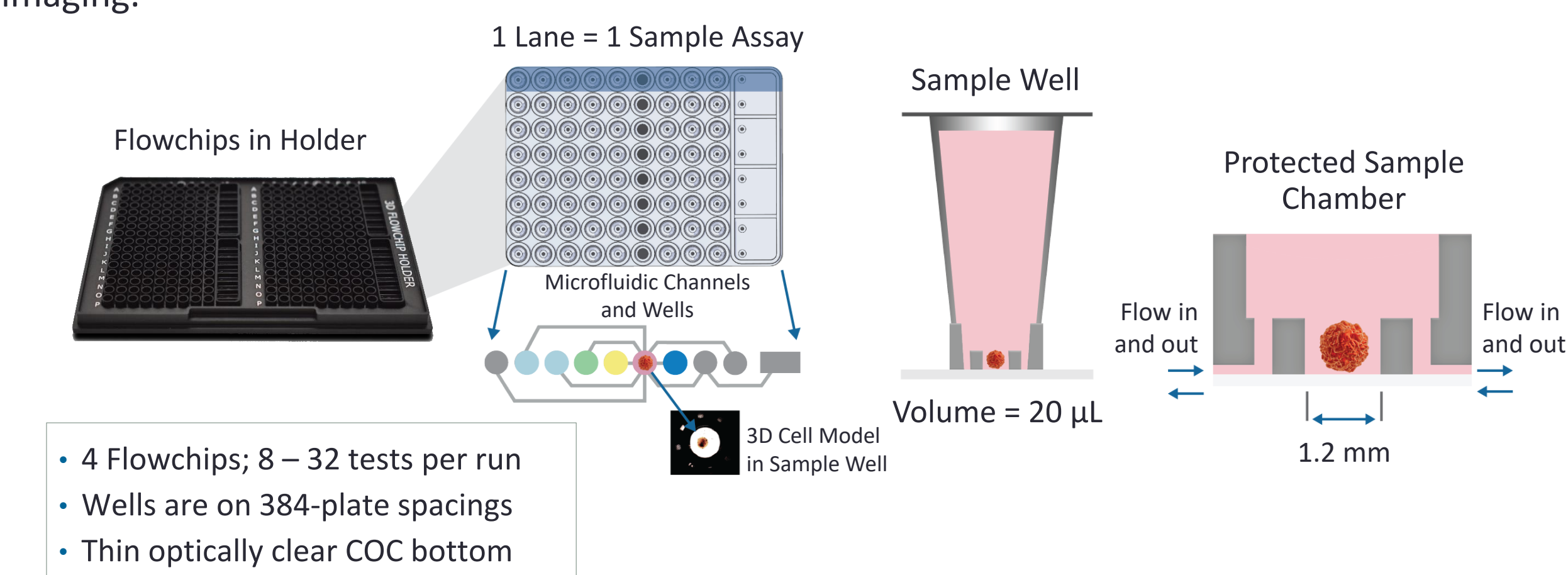


Figure 1. Schematic of Flowchips showing channel layout and sample well with proprietary protected sample chamber. The diameter of the sample well clear aperture is 1.2 mm and compatible with high-content imaging.

References

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3D CO-CULTURE ASSAY WORKFLOW

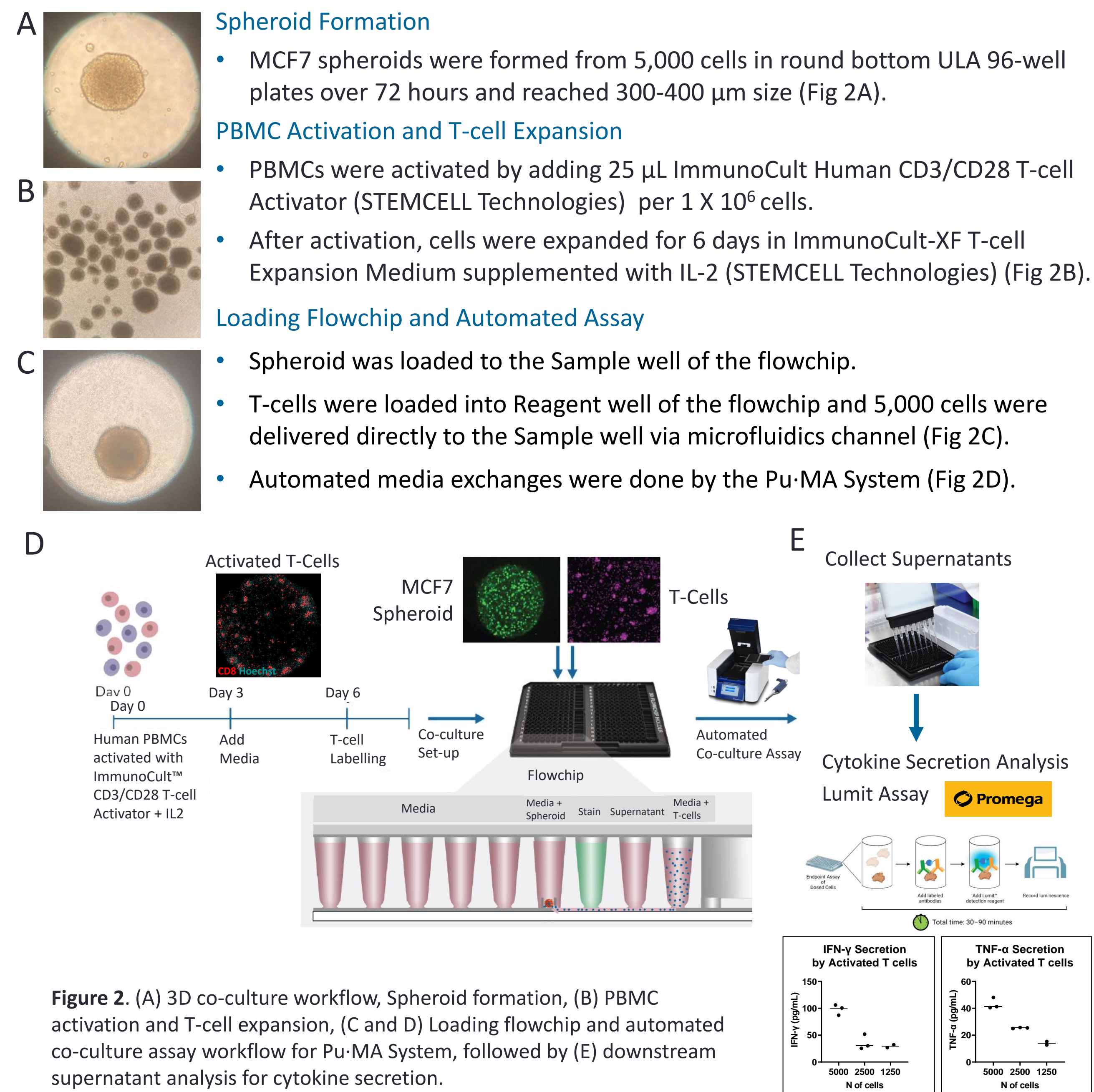


Figure 2. (A) 3D co-culture workflow, Spheroid formation, (B) PBMC activation and T-cell expansion, (C and D) Loading flowchip and automated co-culture assay workflow for Pu-MA System, followed by (E) downstream supernatant analysis for cytokine secretion.

T-CELL CYTOTOXICITY IN CO-CULTURE

- MCF7 spheroids were pre-labeled with CFSE (Carboxyfluorescein succinimidyl ester – non-toxic live cell staining dye).
- Activated T cells were pre-labeled with CellTracker Deep Red Dye (Thermofisher Scientific).
- Spheroids and activated T-cells were co-cultured in the flowchip for 48 hr.
- Confocal images were acquired in the flowchips using CellVoyager CQ1 High-Content Analysis System. A 150-200 µm z-stack of images with a 5 µm z-step was acquired using 488 and 561 nm channels.

Maximum intensity projection (MIP) images were acquired at 4 hours and 48 hours of co-culture, along with controls (either T-cells or the MCF7 spheroid alone, Fig 3). At the beginning of co-culture, the MCF7 spheroid (green) displayed a compact and round shape. The spheroid was surrounded by clusters of activated T-cells (magenta). Upon interaction of cancer spheroid and T-cells we observed T-cell redistribution and localization within the spheroid. The T-cell effector activity resulted in spheroid loss of shape and integrity with increased presence of damaged and dead cancer cells. The control spheroid (no T-cells exposure) maintained its shape, architecture, and remained intact (Fig 3).

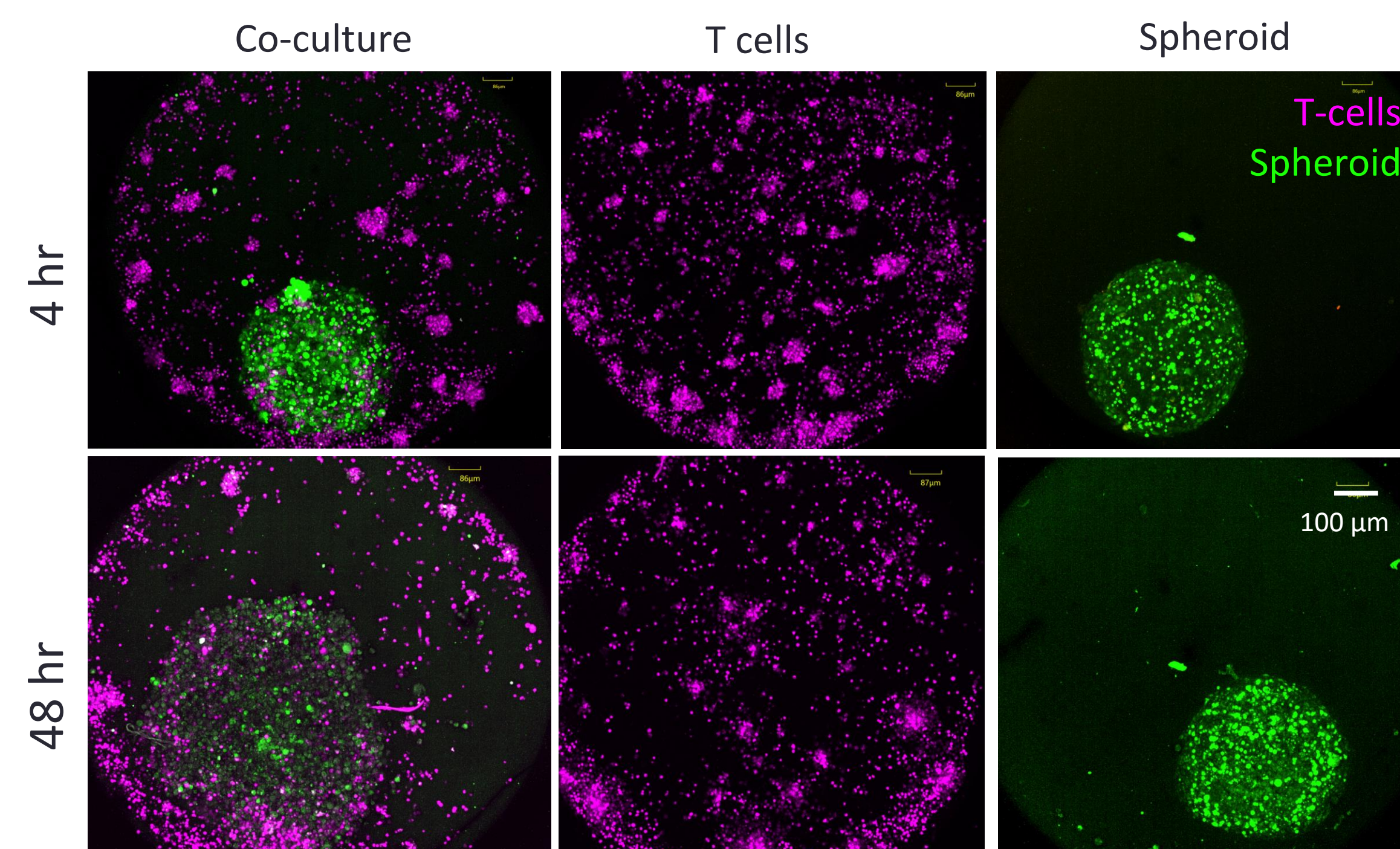


Figure 3. T-cell – MCF7 spheroid interaction over 48 hours in co-culture (Confocal MIP images)

AUTOMATED IF STAINING FOR PHENOTYPIC PROFILING

Automated IF staining of fixed samples was performed in Pu-MA System at room temperature, workflow shown in Figure 4.

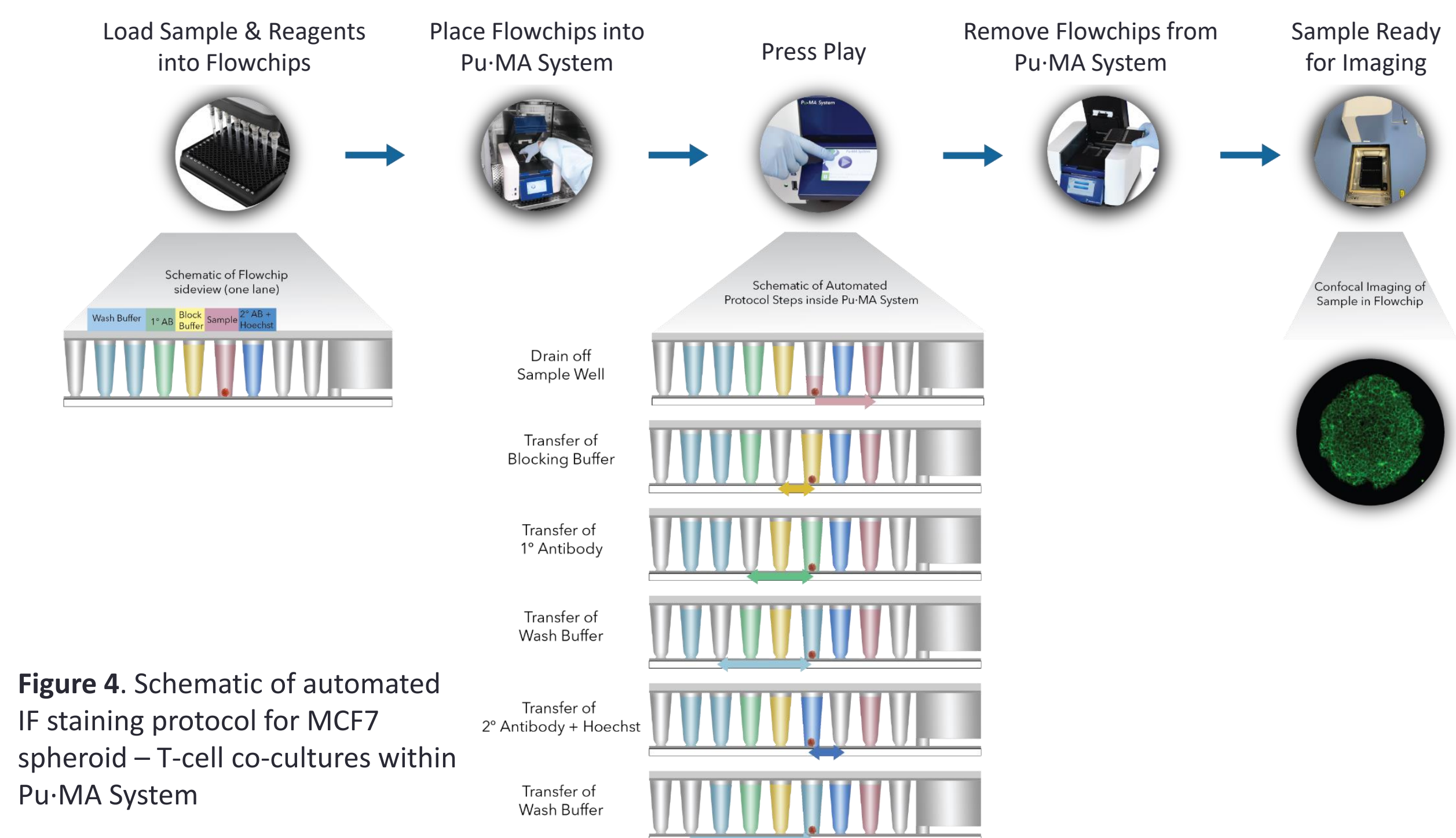


Figure 4. Schematic of automated IF staining protocol for MCF7 spheroid – T-cell co-cultures within Pu-MA System

PD-L1 EXPRESSION IN BREAST CANCER 3D MODELS

- TU-BcX-41C Tumoroids**
- Derived from a primary TNBC tumor
 - Highly metastatic, rapid growth
 - Aggressive and resistant to therapy
- MCF7 Spheroids**
- ER Positive
 - Non-aggressive and non-invasive
 - Low metastatic potential

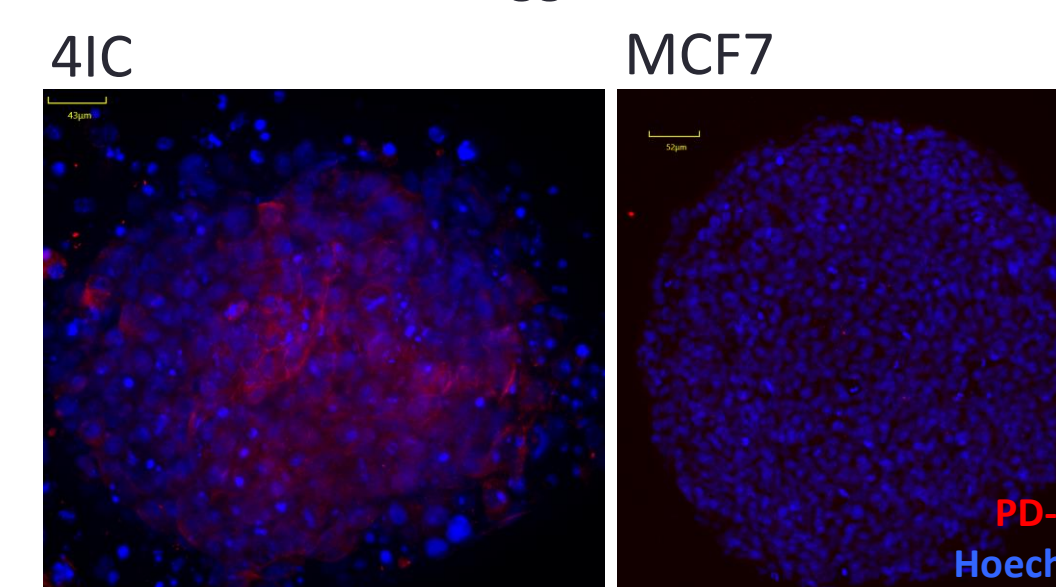


Figure 5. PD-L1 expression in 41C tumoroids and MCF7 spheroids (Confocal MIP images)

Pre-formed 41C tumoroids and MCF7 spheroids were fixed, washed, and stained for PD-L1 using automated IF staining PuMA System protocol.

MCF7 spheroids and 41C tumoroids demonstrated differential expression level of PD-L1.

The automated co-culture platform will be further used to assess how PD-L1 status, and its modulation affect T-cell cytotoxic activity.

T-CELL PHENOTYPING IN 3D CO-CULTURE

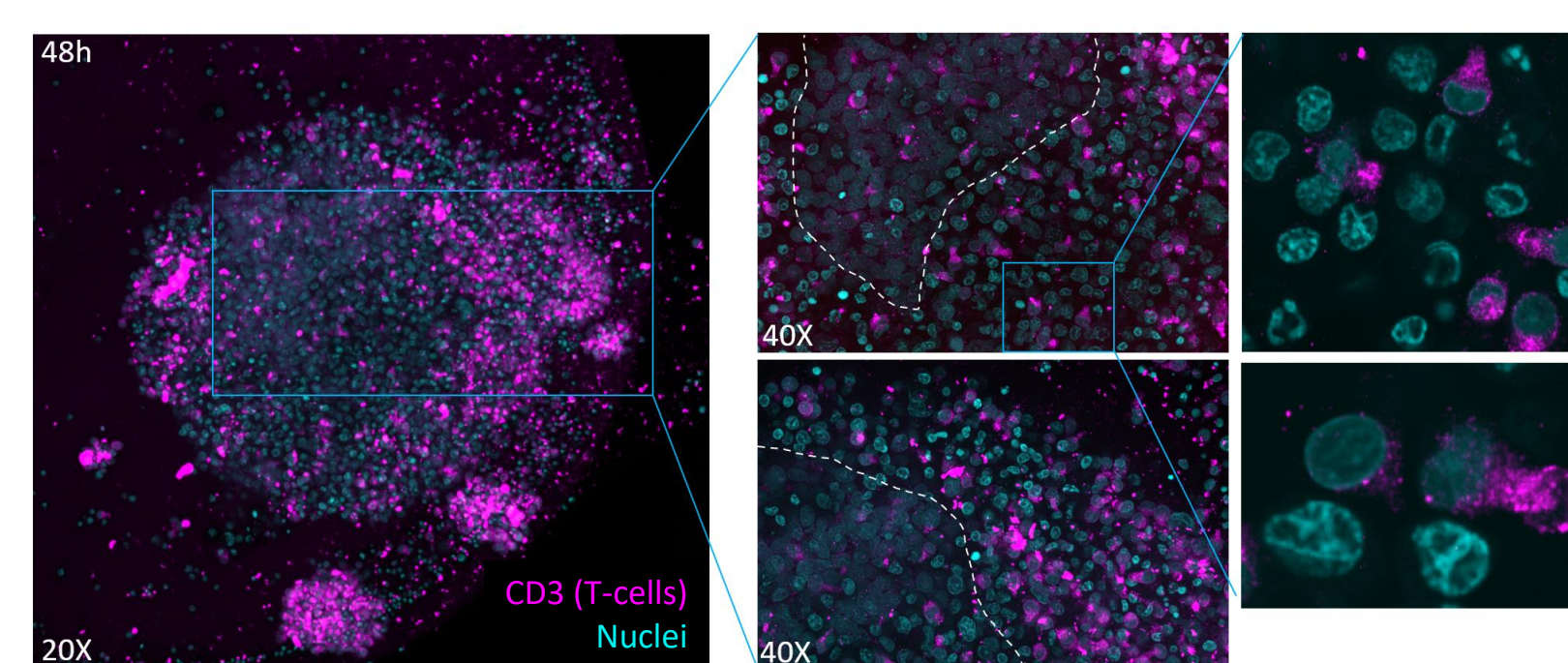


Figure 5. T-cell – MCF7 spheroid interaction over 48 hours in co-culture. (Confocal MIP images)

Automated IF staining of 48-hour co-cultures demonstrated significantly more T-cells present at the edge of spheroid and they were associated with significant cancer cell damage (Fig 5).

The area closer to the middle of spheroid showed presence of fewer T-cells and more intact spheroid architecture with healthier looking cells compared to peripheral areas.

CONCLUSIONS

- In this study we demonstrated how immune-cancer cell co-culture assays can be streamlined using automated Pu-MA System.
- 3D co-culture platform holds great value for studying how PD-L1 modulation affect T cell cytotoxic function.
- Automated immunofluorescence staining protocol, combined with high-content imaging, allows deeper studying the interplay between tumor microenvironment components and different tumor infiltrating immune cell populations, their spatial organization features.
- 3D platform allows to study the crosstalk between immune, cancer and other cell interactions, evaluate new drug candidates and assess individual therapeutic approaches to advance precision medicine.

