# A novel workflow to assess the T-cell and patient-derived organoid interaction

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## Introduction

### T-cell CRC PDO interaction assay

Immunotherapy is increasingly popular as a type of cancer treatment. These therapies include the use of Chimeric Antigen Receptor-engineered T-cells (CAR T-cells), tumor-infiltrating lymphocytes (TIL), and other genetically modified T-cells to specifically target the cancer cells. Although much success has been achieved with immunotherapy for treatment of blood cancers, its efficacy remains limited in solid tumors. One of the reasons for the low success rate is attributed to the solid tumor microenvironment (TME) where suppressive cytokines limits the tumor killing ability of T-cells. Thus, understanding the role the TME plays in T-cell responses is essential for the development of effective cancer therapies.

The benefits of using three-dimensional (3D) patient-derive organoids (PDOs) lie in the physical and chemical cues present within the TME that cannot be mimicked in traditional 2D monolayer cultures. Studies show that PDOs show similar responses to drugs as original tumors, suggesting the value of using PDOs to improve therapeutic outcomes. Thus, PDOs can provide more relevant physiological and pathological cancer models that recapitulate the basic features of primary tumors and is more suited for assessing the effectiveness of T-cell killing than 2D cell models.

Despite the benefits associated with the use of PDOs, there are significant barriers to widespread adoption of PDOs in drug discovery. Organoid production is a costly and highly labor-intensive process. Moreover, organoid culture is a skilled manual process, and therefore can vary significantly between operators. To address the challenges associated with the use of PDOs in large scale applications, a semi-automated bioprocess has been developed for the large-scale expansion of assay ready organoids. Here, we developed a method to assess the effectiveness of T-cell invasion in solid tumors using PDOs. Using bioreactor expanded patient-derived colorectal cancer orgnoids (CRCs) (Molecular Devices), activated human peripheral blood mononuclear cells (PBMCs) stained with CellTracker were added to CRCs (stained with MitoTracker) in a 96-well microtiter plate and monitored every 4 hours for 3 days using high-content imaging. To quantify T-cell invasion, we developed an image analysis method to measure the distance of each T-cell to the nearest organoid (a distance). We found that stimulated T-cells resulted in smaller interaction distance than non-stimulated T-cells. The results demonstrate the utility of the bioreactor-expanded organoids in large scale T-cell-based screens.

## Methods

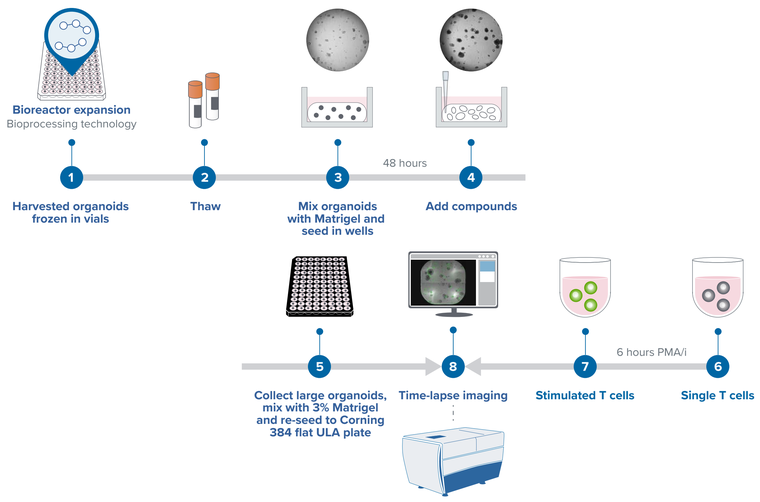


**Figure 1.** T-cell and CRC PDO interaction workflow

- Bioreactor-expanded patient-derived colorectal cancer organoids (CRC) were first mixed with 80% Matrigel and grew 48 hours before collection. The collected large organoids were then stained with MitoTracker Red and mixed with 3% Matrigel before seeding into 384-well flat-bottom plates with ultra-low attachment (Corning). The thawed PBMC/T-cells were stimulated in PMA/i for 6 hours and stained with CellTracker Green before being added to the CRC organoids for co-culture.
- We used the ImageXpress® Confocal HT.ai High-Content Imaging System (Molecular Devices) equipped with spinning disk confocal and sCMOS camera to perform timelapse live imaging every 4 hours.

## Automation setup

An automated workcell consisting of an incubator and a high-content imager (green box in Figure 2) was used to monitor the co-culture of organoids and T-cells. The Genera scheduling software (RETISOFT) was used to execute routine organoid monitoring in culture. The protocol involves the retrieval of the plate from the incubator, transport of plate to the ImageXpress Confocal HT.ai to image the organoids every 4 hours (z-stack acquisition, 10X), and placing the plate back in the incubator using the PreciseFlex400 robotic arm (Brooks).

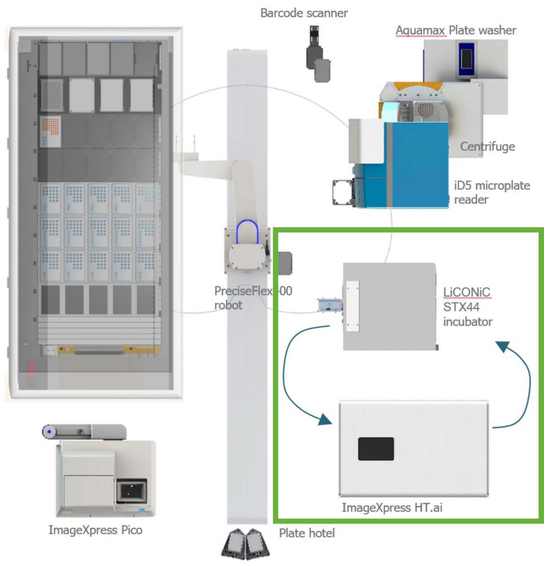


**Figure 2.** Layout of the automated workcell. A standard protocol consists of a liquid handler (Hamilton), a robotic arm (Brooks), incubator (LiCONiC), ImageXpress HT.ai confocal imager (Molecular Devices), ImageXpress Pico Automated Cell Imaging System (Molecular Devices), iD5 Multi-Mode Microplate Readers (Molecular Devices), AquaMax Microplate Washer (Molecular Devices), a plate hotel, a centrifuge, and a barcode scanner. The curved arrows show an example of the process to monitor cells in culture where plates are moved from the incubator to the ImageXpress Confocal HT.ai for imaging and then back to the incubator.

## Assay optimization

To improve access of T-cells to Matrigel-embedded organoids, we sought to determine the optimal Matrigel concentration for the assay. Ideally, the amount of Matrigel used should be sufficient to maintain the CRC structure while allowing T-cells to migrate freely towards the organoids. CRC organoids in 80% Matrigel continue to increase in size while those embedded in 3%, 5% or 10% Matrigel show no significant change in size. Interestingly, CRC organoids in media only show slight increase in size. This could be due to the effect of the biophysical properties of Matrigel (such as stiffness) on organoid morphology[1]. From the results, we chose to use 3% Matrigel to maximize T-cell penetration efficacy while maintaining the overall organoid structure.

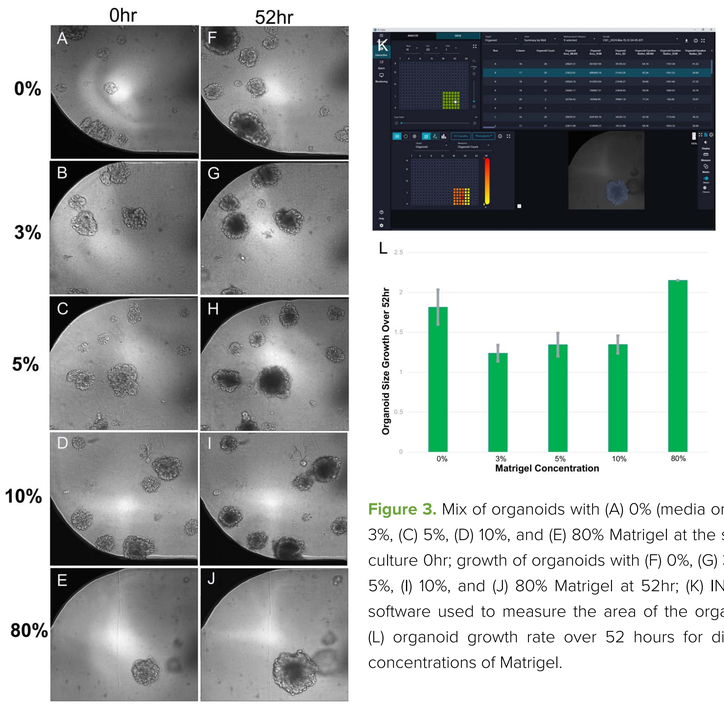


**Figure 3.** Mix of organoids with (A) 0% (media only), (B) 3%, (C) 5%, (D) 10%, and (E) 80% Matrigel at the start of culture 0hr; growth of organoids with (F) 0%, (G) 3%, (H) 5%, (I) 10%, and (J) 80% Matrigel at 52hr; (K) IN Carta software used to measure the area of the organoids; (L) organoid growth rate over 52 hours for different concentrations of Matrigel.

## Results

### T-cell reciprocal interaction with PDO

We observed that T-cells and organoids displayed varying degrees of motility in culture. Figure 4 shows the progressive reciprocal interaction between T-cells and CRC organoids. Stimulated T-cells tend to accumulate around the organoids (Figure 4A–4F), while non-stimulated T-cells do not. Compared to the non-stimulated condition, where the relative position of organoids remain unchanged, stimulated T-cells appear to displace organoids in culture (see white arrows in Figure 4B and 4F).

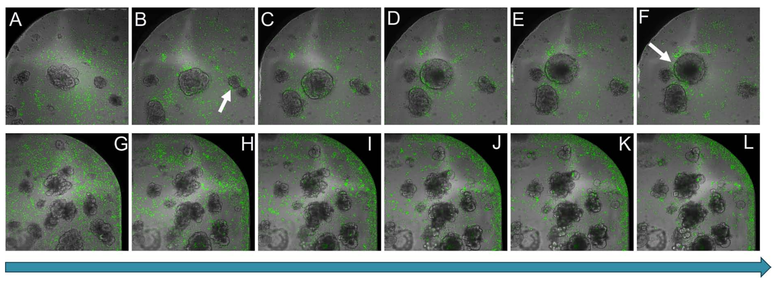


**Figure 4.** (A)–(F) Stimulated T-cells were accumulated around the edge of the organoids and attracted the organoids together; (G)–(L) non-stimulated T-cells were accumulated around the edge of the wells without affecting the organoids.

### Edge accumulation analysis

To characterize the effects of stimulated T-cell accumulation on the organoids, we first measure the distance from each T-cell to the nearest organoid (where T-cells inside the organoids were not considered) (Figure 5A–5F), using custom module editor (CME). If we consider 50 µm as the interaction distance between T-cells and organoids and average all the T-cell distances within 50 µm from all the wells with the same treatment, we find that the stimulated T-cells tend to localize to the edge of the organoids, which results in a reduced average distance across the culture time. It saturates at around time point 6 (24hr after treatment). The non-stimulated T-cells accumulate around the edge of the wells and the average distance also decreases in some extent, but more mildly than the stimulated T-cells.

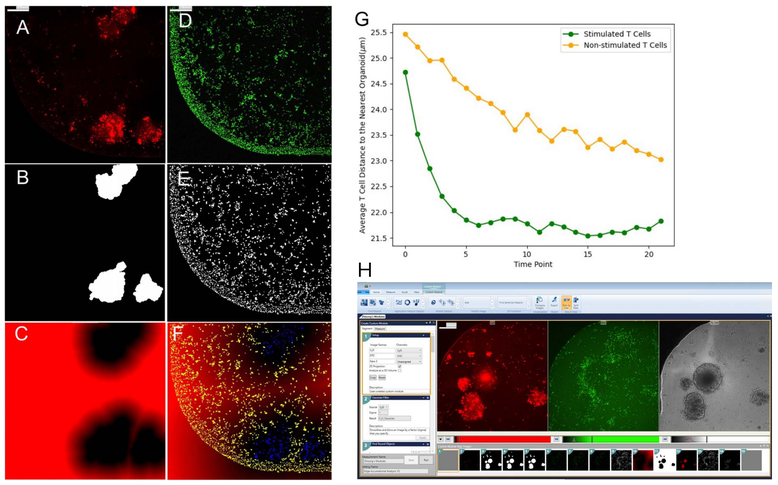


**Figure 5.** (A) Mito-tracker Red staining mitochondria in the organoids; (B) the organoid masks generated from mito-tracker staining, indicating the boundary of each organoid; (C) 16-bit grayscale image with pixel value representing the distance to the nearest while pixel in (B); (D) cell-tracker Green staining T-cells; (E) the T-cell masks generated from cell-tracker staining; (F) the distance of T-cell to the nearest organoid is measured by overlaying (C) and (E); (G) the average distance of all T-cells from all wells with the same treatment (10 wells for stimulated, 5 wells for non-stimulated) across all time points. (H) the custom module editor used to analyze the T cell distance.

## Summary

- We used time-lapse high-content imaging to monitor T-cell and organoid interaction using the ImageXpress Confocal HT.ai High-Content Imaging System.
- We used the custom module editor to analyze the T-cell accumulation on the organoid edges.
- We proposed the method to measure the interaction between T-cells and the organoid.

### Reference

1. Borries, M.; Barooji, Y.; Yennek, S.; Grapin-Botton, A.; Berg-Sørensen, K.; Oddershede, L. Quantification of Visco-Elastic Properties of a Matrigel for Organoid Development as a Function of Polymer Concentration. *Front. Phys.* 2020, 8, 579168