Optical coherence tomography (OCT) for label-free real 3D imaging of ex-vivo tumor cultures

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Introduction

The use of advanced *ex-vivo* human organotypic cultures is rapidly growing in the field of oncology research and diagnostics, with a focus on patient-derived organoids and tumor tissue specimens cultured in artificial systems capable of replicating tumor development mechanisms. These mechanisms include metastasis, angiogenesis and formation of dysplasia. Our objective is to enhance the label-free imaging and analytical capabilities of these complex tissue samples to enable screening and diagnostics applications.

SCREEN Holdings co. ltd, has developed a unique infrared laser-based optical coherence tomography (OCT) technology enabling non-invasive, label-free, three-dimensional (3D) imaging of tumoroids, epithelial cystic organoids, sprouting endothelial neo-vasculature and metastatic single cells. The imaging is carried out on the 3DProSeed hydrogel plates developed by Ectica Technologies, a glass-bottom 96-well plate featuring pre-casted, synthetic and optically clear hydrogels for ex-vivo tumor cultures.

The 3DProSeed hydrogel plates offer the highest workflow integration in screening processes. No hydrogel assembly step is necessary: the hydrogels are pre-casted in the plate and delivered hydrated and ready for cell seeding. Thanks to the patented hydrogel surface, no cell encapsulation procedures are required. Additionally, the hydrogels, made of poly(ethylene glycol)-based bioconjugates, are fully synthetic and animal free and offer the highest control over the culture conditions, as well as the possibility to upgrade to GMP for cell therapy applications. Finally, various cell populations can be sequentially seeded at different time points to generate complex co-cultures. In this way, stromal environments can be created under controlled conditions, to which cancer cells can be subsequently added.

Here we present culture and imaging protocols with the resulting 3D tomographic reconstructions of endothelial sprouting vessels, cystic epithelial organoids of the colon and 3D invasion assays of highly metastatic glioma cells.

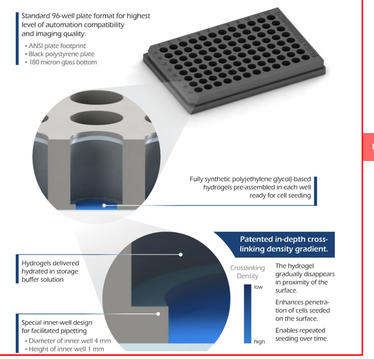
Organotypic cell culture and optical coherencetomography (OCT) imaging workflow

Samples

- Tissue specimens
- Organoids
- Primary cells
- Cell lines

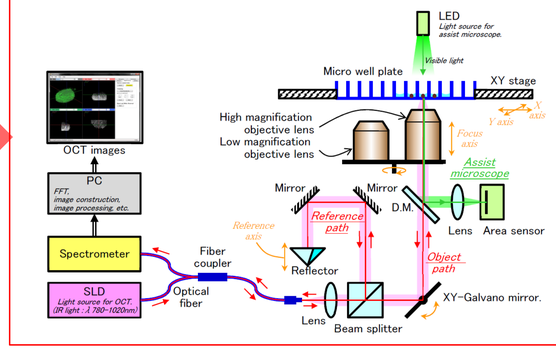
Direct seeding on hydrogel plate

Culture on 3DProSeed hydrogel 96-well plates



Imaging

OCT imaging technology on the SCREEN imager



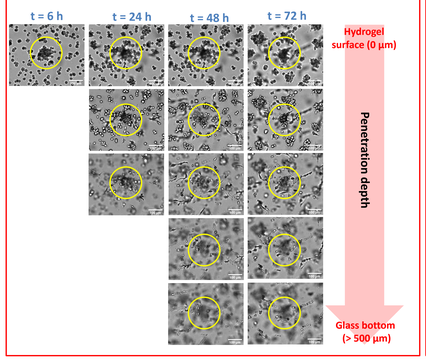
Applications

- Cancer cell 3D invasion assays
- Imaging of organoids in co-culture with stromal cells
- Development of bone marrow vascular niche models

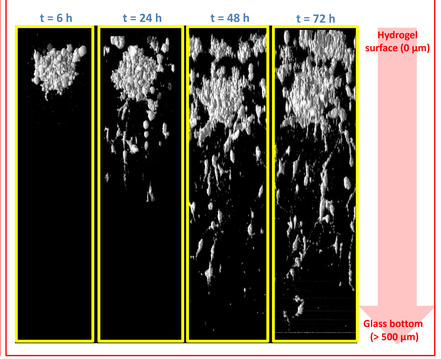
Development of cancer cell 3D invasion assays

At the onset of metastasis, cancer cells must invade through the surrounding tissue and extracellular matrix. The combination of the Ectica hydrogel plates and the Screen OCT imaging system provides an optimal solution to assess 3D cancer cell invasion in a label-free manner. To demonstrate this principle, we used the highly invasive glioblastoma U-87 MG cell line and followed by OCT the migration of individual cells from large clusters seeded at the surface of the hydrogel. Single cell penetration onto the hydrogel over time is evident from bright field images showing cells at different focal planes. However, quantification is very difficult without any cell labeling. On the contrary, 3D OCT data can be easily segmented and the number of cells within a set size window counted throughout the hydrogel volume.

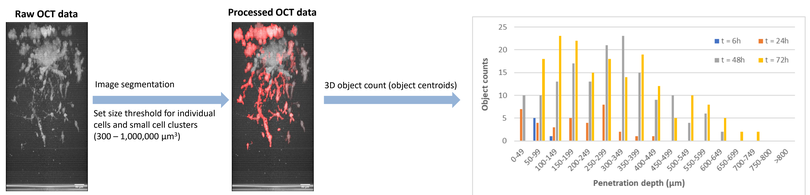
Bright field imaging: individual z slices



OCT imaging: 3D reconstructions



OCT imaging: Quantification of cell invasion

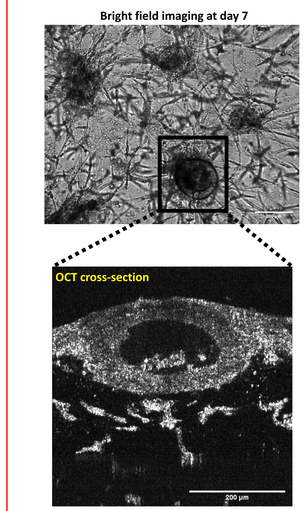


- Glioblastoma cancer cell clusters can be seeded on the hydrogel surface and their migration through the gel can be monitored by different imaging modalities.
- OCT imaging allows quick image segmentation, 3D reconstruction and quantification of the invading cells in a label-free manner.
- This assay can be extended to other invasive cancer cells, including patient-derived cells and patient-derived organoids.

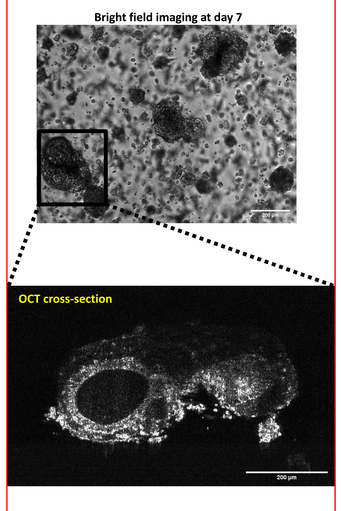
Development of colon organoid and stromal cells co-cultures

Colon organoids derived from induced pluripotent stem cells (iPSC) have proven to be a powerful *ex vivo* tool for research into colon cancer. However, many of the current organoid culture platforms do not consider the effect of the stromal compartment on the development and function of the organoids. Here, we demonstrate the capability of our hydrogel plates to support organoid growth on two different stromal environments: adipose-derived mesenchymal stromal cells (Ad-MSC) and enteric glial cells (EGC). Ad-MSC form an extensive cellular network onto which the organoids become enmeshed, whereas EGC develop into a disperse culture of sparse cells and do not come into close contact with the organoids. The co-cultures can be subjected to OCT imaging and from the resulting 3D reconstructions one can perform a morphometric analysis of the different types of organoids.

Colon organoid culture on Ad-MSC stroma



Colon organoid culture on EGC stroma

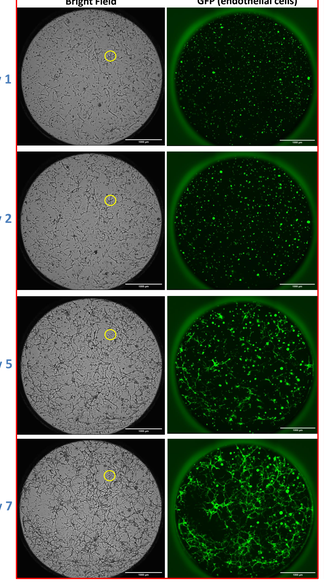


- Colon organoid cultures can be established in hydrogel plates pre-cultured with different types of stromal cells.
- The organoid-stroma co-cultures are amenable to OCT imaging to generate label-free 3D reconstructions of the organoids that can be used to extract morphometric parameters, in particular of the luminal structure not clearly visible using light transmission imaging.
- This platform can be extended to cancer patient-derived organoids as well as iPSC-derived organoids spiked with cancer cells.

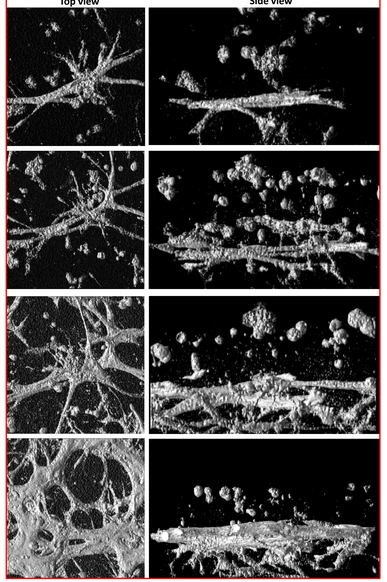
Development of bone marrow vascular niche models

The bone marrow vascular niche is essential for the development and maintenance of the hematopoietic system and plays critical roles in blood cancers. To understand these roles and use them to our advantage to develop effective cancer therapies, we need *ex vivo* models that mimic as much as possible its composition and architecture. Here, we seeded GFP expressing bone-marrow endothelial cells (BMEC1) onto bone-marrow derived stromal cells (BM-MSC) cultured already on the hydrogel plate, and we followed the development of an extensive 3D vascular network over the course of 7 days. A selected area, indicated by the yellow circle in the bright field images, was also followed by OCT imaging, which allowed the 3D reconstruction of the cellular network and revealed the development of a tight interaction between the endothelial and stromal cells.

Optical microscopy: whole-well overviews



OCT imaging: 3D reconstructions of zoomed areas



- Extensive 3D vascular networks can develop in hydrogel plates pre-cultured with bone-marrow stromal cells.
- OCT can offer insights onto the dynamics of the co-culture: the endothelial cells migrate through the gel towards the stromal cell network and develop tight association with them.
- Such models can find applications in drug screenings to assess the role of the bone marrow vascular niche in cancer therapy.

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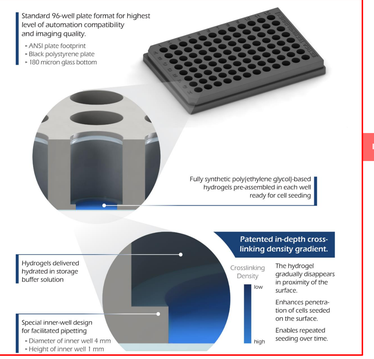

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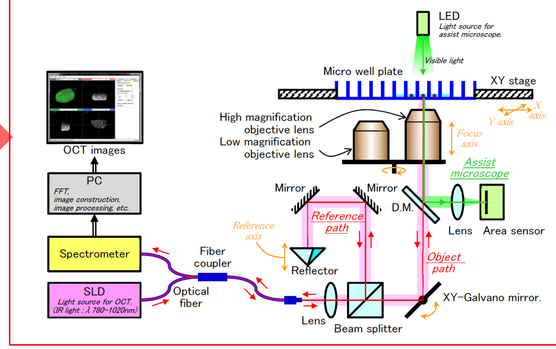

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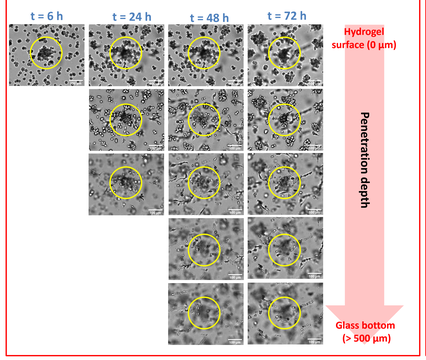

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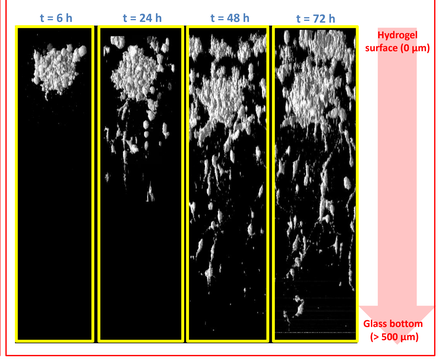

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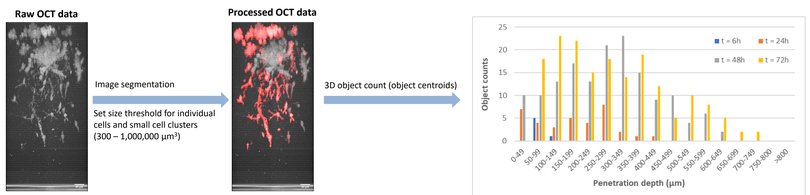

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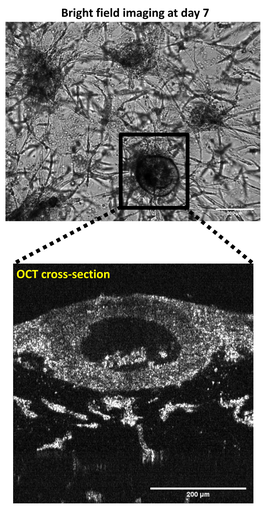

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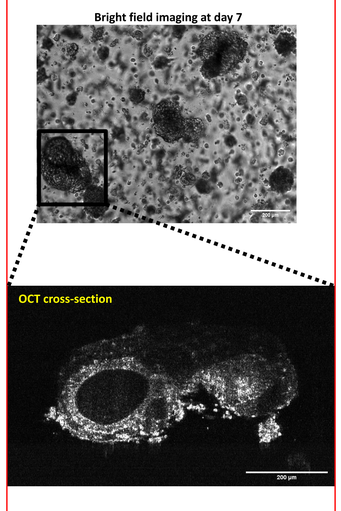

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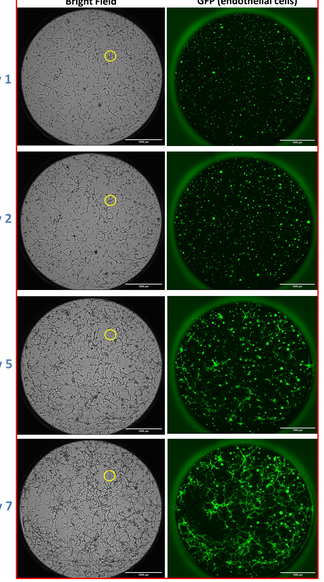

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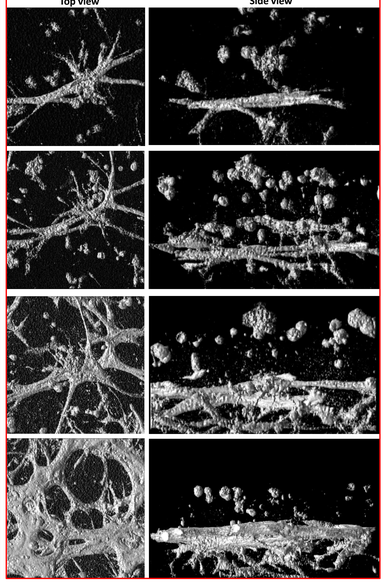

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