DEVELOPMENT OF A HIGH CONTENT SCREENING APPROACH ON 3D HUMAN COLON ORGANOIDS TO IDENTIFY DRUG CANDIDATES AGAINST COLON CANCER

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The recent technological advances emanating from both microscopy and computing have enabled the development of the **High Content Screening (HCS)**. HCS is based on **automated microscopy**, **multi-parametric image processing** allowing High Content Analysis (HCA) to **get quantitative information** from organoid and cell populations. HCS thus represents an early drug-discovery platform helping to define the functions of genes, proteins and other biomolecules in normal and pathological cell functions.

Here, we present the development of a high content imaging assay to screen drug candidates against colon cancer stem cells based on **3D human organoids model**. This model is generated from epithelial stem cells isolated from either normal crypts or tumoral tissue resections from patients with colorectal cancer (CRC) or inflammatory bowel disease (IBD).

Until now, colon organoid model is characterized at different time points using classical and confocal microscopy on fluorescent-labelled cultures. However, this sequential approach does not allow to keep the cultures alive at long term, is highly time-consuming due to the slow acquisition speed with conventional confocal imaging and, besides, the analysis is manual and user-dependent.

In this study, we develop a High Content Screening approach, allowing simultaneous acquisitions in a large Z-stack (with or without fluorescent-labelling) coupled to image analysis based on a maximum intensity projection of z-stack to **recognize, count, classify and follow up the 3D colon organoid cultures**. Then, we investigate the effects of thrombin and its inhibitor on the phenotype, differentiation and cellular apoptosis of 3D model from healthy patients.

To sum up, we have established a fast and robust following-based HCS approach on 3D model. This will lead to a better understanding of the colon pathophysiology as well as the identification of new drug candidates in CRC and IBD.

Further orientation
Sirenko O, Mitlo T, Hesley J, Luke S, Owens W, Cromwell EF. High-Content Assays for Characterizing the Viability and Morphology of 3D Cancer Spheroid Cultures. *Assay and Drug Development Technologies*. 2015;13(7):402-414.