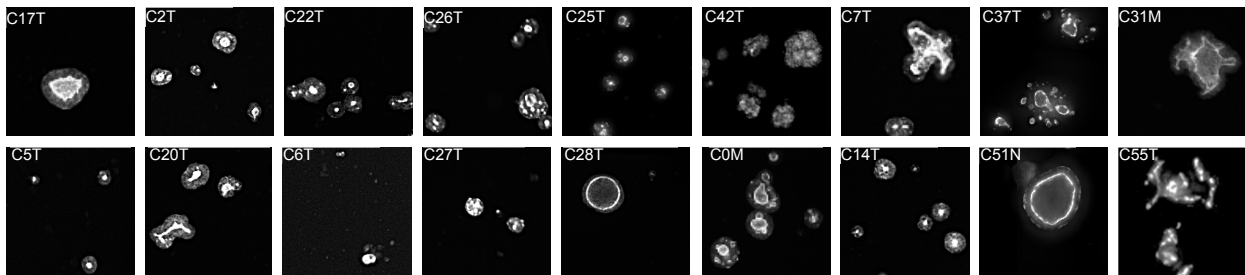


Morphology-based screening of CRC organoids

Colorectal cancer-derived organoids are polarized epithelial micro-tissues that are derived from resected CRC patient material and cultured in a 3D matrix in the presence of stem-cell stimulating factors to sustain ex vivo growth. The Organoid Technology, developed by the **Hubrecht** institute in Utrecht (NL), allows propagation of healthy (normal) colon organoids and tumor-derived organoids, or tumoroids. By including a large patient population, a diverse, representative living biobank of colon organoids is generated by the HUB foundation, genetically characterized by the **Sanger** Institute (UK) and morphologically screened by **OCellIO** (NL) to functionally annotate bispecific stem-cell targeting antibodies produced by **Merus** (NL) to treat colorectal cancer (together with **IRB** (SP), SuppresSTEM).



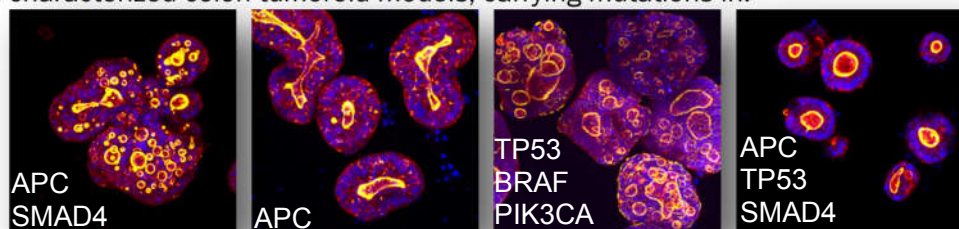
At OCellIO we use Hubrecht Organoid Technology to culture and screen tissues from patient-derived colon tumor and normal cells in a physiologically relevant microenvironment. A dedicated automated image acquisition, -storage, **-3D analysis and morphological annotation pipeline** was developed to measure the responses induced by treating organoids with compounds and antibodies in 384-wells plates in a high throughput format. OCellIO's advanced 3D image analysis enables discrimination of active, selective or cytotoxic molecules.

Key advantages:

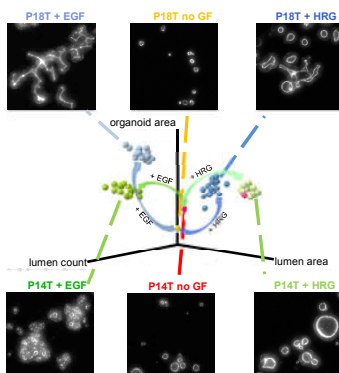
- 3D high content imaging for sensitive detection of therapeutic responses
- Functionally identify inhibitors of CRC tumor outgrowth and development
- Automated high content compound screening in 384-well plate format
- Sensitive and robust measurement of clinically-relevant end-points
- Test mode of action, activity, toxicity and synergy in the same 3D assay

Choose from 80+ well-characterized colon tumoroid models, carrying mutations in:

APC	81%
TP53	69%
KRAS	41%
PIK3CA	22%
BRAF	10%
POLE	6%



Phenotypic Profiling in Colon Tumoroids Cultured in Natural Extracellular Matrix



Patient derived colon tumoroid models, representing diverse well-characterized CRC subtypes, can be grown in 3D and exposed to various drug treatments in diverse growth factor environments. This influences how they grow, develop and form one or multiple lumens at the apical side of the epithelial cell clusters. These morphological changes are exploited to identify the action of antibody or compound treatment on the colon cancer tissues.

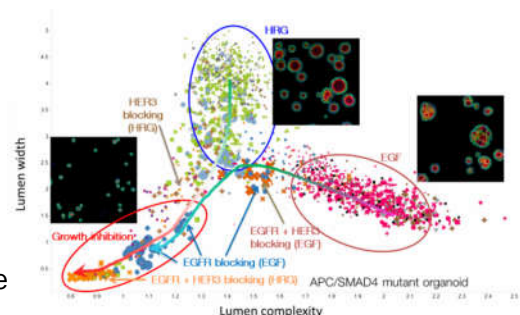
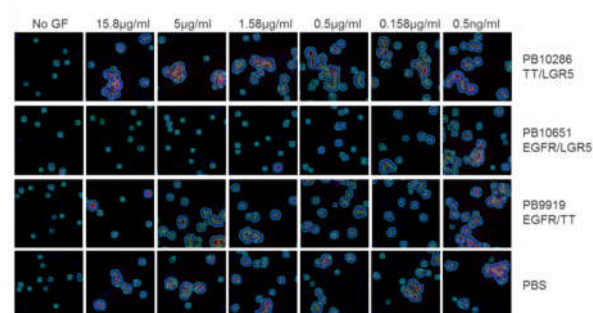


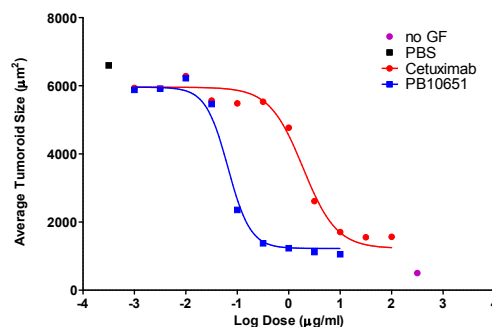
Figure 1 illustrates the workflow of the proposed method for cell segmentation. The process starts with four input channels: DAPI channel (nuclei), Rhodamine channel (organoids), RGB focus on lumen, and Overlay of segmentation. These are processed to generate masks: Nuclei mask, Organoid mask, Lumen mask, and Single cell mask. The Nuclei mask is further processed to show Nuclei mask with single nuclei. The Organoid mask is processed to show Organoid. The Lumen mask is processed to show Lumen branching. The Single cell mask is processed to show Lumen complexity mask. The final results are shown in the bottom row.

Upon 3D culture and compound or antibody incubation, CRC tumoroids are fixed, stained with fluorescent dyes, and image stacks are obtained. A 3D reconstruction of the micro-tissues is generated: typically 150 organoids are obtained per well in a 384 well plate. OCellO's proprietary software evaluation delivers many features, such as: number, shape and size - of organoids, of individual cells and of nuclei; as well as basic measurements of proliferation, apoptosis, and tissue and disease-specific features are collected. Intricate analysis as: network formation, spikes, protrusions, lumen formation and planar polarity are performed in differentiated tumors. These detailed insights enable accurate compound or antibody profiling.



A panel of 545 antibodies was generated by Merus to inhibit the growth factor receptors EGFR or HER3 in combination with any of the four stem-cell surface proteins active in the WNT-signaling pathway, LGR4, LGR5, RNF43 or ZNRF3. These dual targeting bispecific antibodies were screened using OCellio's morphology-based 3D analysis methods in three colon

tumoroids with different mutational background and 52 highly inhibitory bispecific combinations were re-screened in 24 additional CRC organoids. The data demonstrate that **PB10651, a bispecific antibody which targets LGR5 and EGFR, is highly potent in inhibiting colon tumor growth** and has a 20 to 200-fold more potent effect than the clinically approved EGFR-targeting comparison, Cetuximab.



- OCellO's Organoid screening platform enables high throughput testing of compounds in extracellular matrix-embedded tumor cultures;
- 3D-cultured colon tumoroids have proven to show *in vivo*-like growth characteristics and responses to therapeutics;
- Together, the SuppresSTEM consortium has enabled the set up and validation of 3D cultured patient-derived organoid-based screening assays useful for screening of large panels of antibody candidates and identified a highly active bispecific antibody targeting colorectal tumor cells.

Press release (embargo 30th of January 2017)

Ocello licenses Organoid Technology from Hubrecht Organoid Technology to provide commercial screening services for cystic fibrosis and colon cancer

Leiden and Utrecht, The Netherlands; 30 January 2017 – Ocello BV announced today that it has entered into a licensing agreement with Hubrecht Organoid Technology (HUB) for use of Organoid Technology for cystic fibrosis and gastrointestinal diseases, including colon cancer. Ocello will add these organoid assays to its drug discovery services portfolio.

Organoids are mini-organs grown in cell culture from biopsies taken from patients and faithfully recapitulate the genetic and phenotypic characteristics of diseased tissues. Organoids have proven to be valuable disease models for the high-throughput screening and validation of innovative medicines, bypassing limitations associated with cell-line- and xenograft-based preclinical drug studies.

Leo Price, CEO of Ocello, said: “HUB Organoid Technology represent the state-of-the-art for in vitro modelling of drug responses and is expected to form a spearhead in Ocello’s service offering. This licensing agreement is the culmination of a highly successful 3 year EU-funded collaboration (FP7-SUPPRESstem) to develop panels of organoid assays for colon cancer drug discovery and we are delighted to be able to offer these and other organoid assays to our customers.”

Marcel Zwaal, CEO of HUB: “We connect with companies that are able to offer high value added services to our Organoid Technology and with Ocello we have been fortunate to add an expert partner in this field”.

About the HUB

Hubrecht Organoid Technology (HUB) is an organization founded by the Royal Netherlands Academy of Sciences and the University Medical Center Utrecht. HUB exploits the pioneering work of Prof. Hans Clevers, who discovered methods to grow stem cell-derived human ‘mini-organs’ (HUB Organoids) from tissues of patients with various diseases. The organoids, that are part of the HUB biobanks, are characterized by genome sequencing, expression profiling and sensitivity to known and experimental drugs to establish a database linking genetic and transcriptional information to drug responsiveness. HUB offers licenses to its patented HUB Organoid Technology for drug-screening and access to organoids in the HUB biobanks for preclinical drug discovery and validation. In addition, HUB is performing clinical studies to validate the technologies use as a companion diagnostic. More info at www.hub4organoids.eu

About Ocello

Ocello is a CRO that offers drug screening and profiling services using 3D cell culture models of disease. Ocello uses high-content 3D imaging and analysis to measure drug effects, extracting clinically relevant information on compound effects. Ocello has developed a broad range of models for testing potential therapies to treat polycystic kidney disease, neurodegenerative disorders and, in particular, cancer. Ocello’s current 3D cancer models are developed from cell lines, patient derived

xenograft tumours and patient tumour material, and include panels of lung, pancreas, breast, colon and gastric cancers.

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