A microwell platform to standardize human rectal organoid cultures for high-content imaging and phenotypic analyses

Marine Meyer[1], Maria Clapés[1], Angeline Lim[2], Oksana Sirenko[2], Robert Storm[2], Nathalie Brandenberg[1]

[1] SUN bioscience SA Lausanne, Switzerland, [2] Molecular Devices LLC, United States.

In recent years, organoids have emerged as a game-changer for disease modelling and drug screening. These are three-dimensional, miniaturized, and simplified versions of an organ that mimic some of the key features of the native tissue in vitro. Traditional organoid culture methods consist of embedding these structures in solidified extracellular matrix (ECM), creating highly heterogeneous populations. Moreover, organoids are randomly distributed within the ECM which complicates subsequent readouts and images analyses.

To overcome these challenges, we used Gri3D®, a ready-to-use platform for high-throughput and reproducible organoid culture. Based on a standard 96 microtiter plate, each well contains a dense microwell array patterned in a cell repellent hydrogel. The platform enables homogenous cell seeding, efficient cell aggregation and subsequent formation of a single organoid per microwell in suspension-like conditions. A uniquely designed pipetting port, adjacent to each well, allows safe media exchange for long-term cultures. The resulting organoids are positioned in predefined locations in the same focal plane, allowing simultaneous tracking.

Combined with the ImageXpress® Micro Confocal system, we followed the development and self-organization of healthy human rectal organoids over time with brightfield imaging. Using an AI-based approach, we efficiently detected each single organoid and characterized their size, diameter, as well as complex morphological features such as lumen. Finally, we investigated the concentration-dependent toxicity of a small panel of compounds using AI-based brightfield image analyses and fluorescence-based readouts.

The combination of the microwell plates and the high-content imager together with AI-based algorithms allows the assessment of phenotypic features at a single-organoid level in an automatable high-throughput fashion.