3D high-throughput screening of spheroids development using soSPIM

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The recent breakthrough of organoids in the biological research field has offered the possibility to recreate in vitro complex 3D structures closely reproducing morphologies and functions of human organs. Organoids have a strong potential in many fields such as drug screening, toxicity assay, or personalized and regenerative medicine. However, their systematic use still requires to address several challenges in terms of culture standardization, 3D high throughput live imaging, and quantitative analysis.
Indeed, current methods for organoid culture present a significant inter- and intra-batch variability, impacting the robustness of their studies. In addition, the 3D morphologies of organoids and their high sensitivity requires low-phototoxic 3D imaging methods to assess their development in 3D, as well as culture support compatible with 3D advanced imaging methods. In this regard, light-sheet microscopy seems to be the most appropriate technology to image live organoids in 3D, but their multi-objective architecture prevents high throughput screening approaches.

Here we propose to use the single-objective light-sheet microscopy technique soSPIM [1] to overcome these limits. It relies on the use of dedicated micro-fabricated devices integrating 45° mirrors allowing for the creation of a light-sheet perpendicular to the optical axis of the objective. To perform high content imaging of 3D cell cultures, we designed new devices comprising arrays of truncated pyramidal shaped microcavities, called JeWells, compatible with organoids culture and soSPIM imaging. The combination of the JeWells and the soSPIM technology makes now possible to perform typical 3D culture and live imaging in depth of hundreds of standardized organoids in a single well of a classical culture plate.

We will describe our high content 3D acquisition platform, and demonstrate its capacity to monitor live and fixed organoids with unprecedented throughput, and limited photobleaching and toxicity. In particularly, we will highlight the tools we implemented to help for the automatic detection and selection of the positions to image, and ensure a stable and optimal 3D acquisitions on all our positions along acquisition possibly lasting several days.

References:
[1] Galland et al, “3D high- and super-resolution imaging using single-objective SPIM”, *Nature Methods*, 2015