soSPIM image enhancement methods for 3D cell cultures imaging

T. Delaire[1], I. Idrissi[1,2], G. Grenci[3], V. Viasnoff[3], R. Heintzmann[4], R. Galland[1], J.B. Sibarita[1]

[1] Univ. Bordeaux, CNRS, Interdisciplinary Institute for Neuroscience, IINS, UMR 5297, 33000 Bordeaux, France; [2] Sanofi, In vitro Biology/ Integrated Drug Discovery, CRVA, 1 impasse des Ateliers 94403, Vitry s/ Seine, France; [3] Mechanobiology Institute, National University of Singapore, 117411, Singapore; [4] Abbe Center of Photonics, Friedrich-Schiller-University, 07745 Jena, Germany.

Abstract

Over the last decades, 3D cell cultures, with which it becomes possible to reproduce part of the morphology and functions of human organs, have emerged as future gold standards for drug screening and toxicity assays. Investigating the development of these 3D cultures is especially interesting because they closely mimic various behaviours observed in vivo, contrary to their 2D counterparts still massively used nowadays. However, the study of such 3D objects requires the development of dedicated imaging platform allowing their precise characterization at the cellular, multi-cellular and whole object levels.

In that perspective, our team, in collaboration with the MechanoBiology Institute in Singapore, have developed a high-content screening platform based on the single-objective light-sheet microscopy technology (soSPIM) [1,2]. This imaging method relies on micro-fabricated devices integrating 45° micro-mirrors to create a sheet of light perpendicular to the optical axis of the microscope, and to collect the fluorescence with only one objective. Compatible with any inverted microscope, this architecture removes the mechanical constraints of the standard multi-objectives light-sheet microscopes. Combined with new devices, called JeWells, composed of truncated pyramidal shaped micro-cavities, it allows parallelizing and standardizing the culture and imaging of hundreds of 3D samples within a single chip [2]. Despite its efficient optical sectioning and fast imaging capabilities, soSPIM images are limited both in term of contrast and homogeneity, similar to any traditional light-sheet imaging techniques.

We here present several methods allowing drastically improving the soSPIM image quality, such as deconvolution, multi-view image fusion and optical sectioning by structured illumination. We will show how the unique JeWell-soSPIM architecture, relying on truncated pyramidal shaped micro-cavities embedding the 3D cultures, allows to drastically simplify their implementations, which can be optically complex and computationally intensive on traditional light-sheet microscopes. Results of those three approaches will be presented on different 3D culture models.

References

1. Galland et al, “3D high- and super-resolution imaging using single-objective SPIM”, Nature Methods, 2015
2. Beghin et al, “Automated high-speed 3D imaging of organoid cultures with multi-scale phenotypic quantification”, Nature Methods, 2022