High-throughput live imaging using Light Sheet Microscopy

Emilio J. Gualda, Matteo Bernardello, Maria Marsal and Pablo Loza Alvarez
ICFO-Instituto de Ciencias Fotónicas, BIST-Barcelona Institute of Science and Technology, 08860 Castelldefels, Spain

Abstract— We have developed a compact, multi-modal light-sheet microscope platform where different kind of experiments may be addressed with a single equipment. We will evaluate the performance of the Flexi-SPIM platform and its ability to carry out high-throughput quantitative analysis with high resolution, high speed and minimal photo-damage on complex 3D cell cultures and the microtubule network during zebrafish development.

Keywords— Light Sheet Fluorescence Microscopy; High-throughput microscopy; 3D cell culture; zebrafish.

I. Introduction

Light-sheet Fluorescence Microscopy (LSFM) has gained much interest recently due to the low induced photodamage, fast acquisition rates and the possibility to reconstruct high quality images of whole organisms [1]. However, current LFSM microscopes, revealed to be unpractical for large-scale experimental application and high-throughput screens requiring special sample preparation. In the majority of the described systems, samples need to be one by one mounted, embedded in agar gel, limiting the number of samples analysed. In 2015 we created the first flow cytometry system based on LSFM able to handle 3D cell cultures and zebrafish: SPIM-Fluid [2, 3]. The fusion of LSFM and fluidics, allowed massive 3D cell cultures studies and sophisticated cell-based assays in real-time.

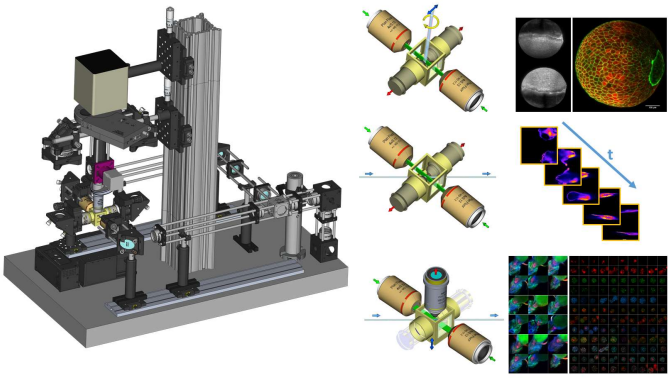

Fig. 1. Scheme of the Flexi-SPIM setup and the three different acquisition modes provided: Classical, Flow and Hybrid LSFM.

Here we present a new modular system architecture, Flexi-SPIM (Fig.1), that overcome limitations of our previous designs at the same time that extend the microscope functionalities, making it ideal for imaging facilities that need to deal with different type of samples and multiple resolution scales. Three different fully automatized acquisition modes are combined on a single machine. Classical LSFM, with mechanical sample scanning and sample rotation; Flow-LSFM with fluidic control of the sample using specialized syringe pumps, which allows high-throughput screens (samples are image as they travels through the channel). Finally, Hybrid LSFM combines fluidic loading of samples and classical scanning, providing high-resolution, multicolour, double side illuminated and single side detected images.

II. Results

Using the Flexi-SPIM system, we are able to make HT quantitative analysis of the three-dimensional organization of the different cell types in 3D cultures [4] with high resolution, high speed and minimal photo-damage (Fig.2). We compared the effect on different cell type combinations over time in order to resemble more realistic 3D cell cultures, and we observed tumour associated macrophage induction and internalization. On zebrafish embryos, we investigated the spatio-temporal organization of the microtubule network during embryonic development, discovering non-reported dynamics.

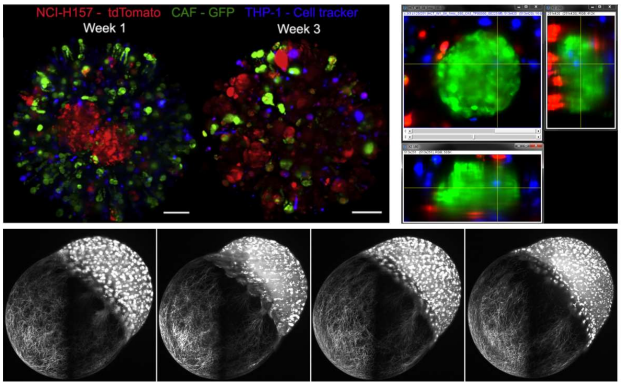

Fig. 2. Maximum projection of two 3D cultures at different time point, and internalization of tumour associated macrophages (top). Dynamics of microtubules at four time points along the zebrafish development (bottom).

Acknowledgment

EJG is supported by MINECO/FEDER projects (SEV-2015-0522 and RYC-2015-17935). MB is supported by Marie Skłodowska-Curie ITN ImageInLife programme.

References

[1] O.E. Olarte et al., Adv. Opt. Photon. 10, 111-179 (2018)
[2] E.J. Gualda et al. Biomed Opt Express; 6, 4447–4456 (2015)
[3] M.F. Estrada et al. Biomaterials, 78:50-61 (2016)
[4] S.P Rebelo *et al.*, *Biomaterials,* 163:185-197 (2018)