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IT-8-P-3023 SPIM-Fluid: High-throughput platform based on Light-Sheet Microscopy

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Drug screens on complex cell models and organisms are a key factor to understand and treat human diseases. However, fast and effective conclusions have been hindered by the lack of robust and predictable models amenable to high-throughput (HT) analysis. Animal models can mimic s pathological features, however species-specific differences may occur and are prone to increase experimental costs. On the opposite side, adherent cell cultures have been used in drug screening and tumor modeling but they do not properly represent biological tissues. Recently, important advances have been made towards the development of 3D cellular models, using human immortalized cell lines, stem cells and other patient derived cells, which better recapitulate features of tissues. These advances bridge the gap between adherent cell culture and animal models, making 3D cellular aggregates an extremely powerful in vitro model for preclinical research.

A major hurdle, hampering the widespread utilization of complex in vitro models, is the lack of robust analytical tools. The development of innovative methodologies will allow more comprehensive readouts, generating more accurate and predictive human cell-based 3D models for drug and toxicity screenings. Imaging techniques like confocal microscopy are not optimal for thick samples, providing a short penetration and long imaging times. As an alternative approach, light sheet microscopy (LSM) has been proposed to overcome those limitations. Novel LSM configurations fusing its inherent capabilities with microfluidics will allow massive live 3D cell cultures studies in real-time and with a high spatio-temporal resolution, enabling sophisticated cell-based assays in 3D cell cultures (disease diagnosis and therapy; drug screening; cell differentiation; etc.). Using this approach we will be able to make HT quantitative analysis of the spatio-temporal organization of the different cell types in a spheroid, as well as the response to different environmental conditions with high resolution, high speed and minimal photo-damage.

We will present new designs and prototypes, and how the use of 3D-cell cultures and full system automation will contribute to measure a large set of biological parameters with statistical relevance to investigate drug response on the central nervous system (CNS), cancer therapy and cell differentiation. Also, it would facilitate the development of new typologies for 3D-cell cultures and optimize staining protocols. Those systems will be primarily devoted to 3D cell cultures studies, but the expansion to other biological systems, such as full brain imaging in zebrafish embryos with cellular resolution, will be also presented.

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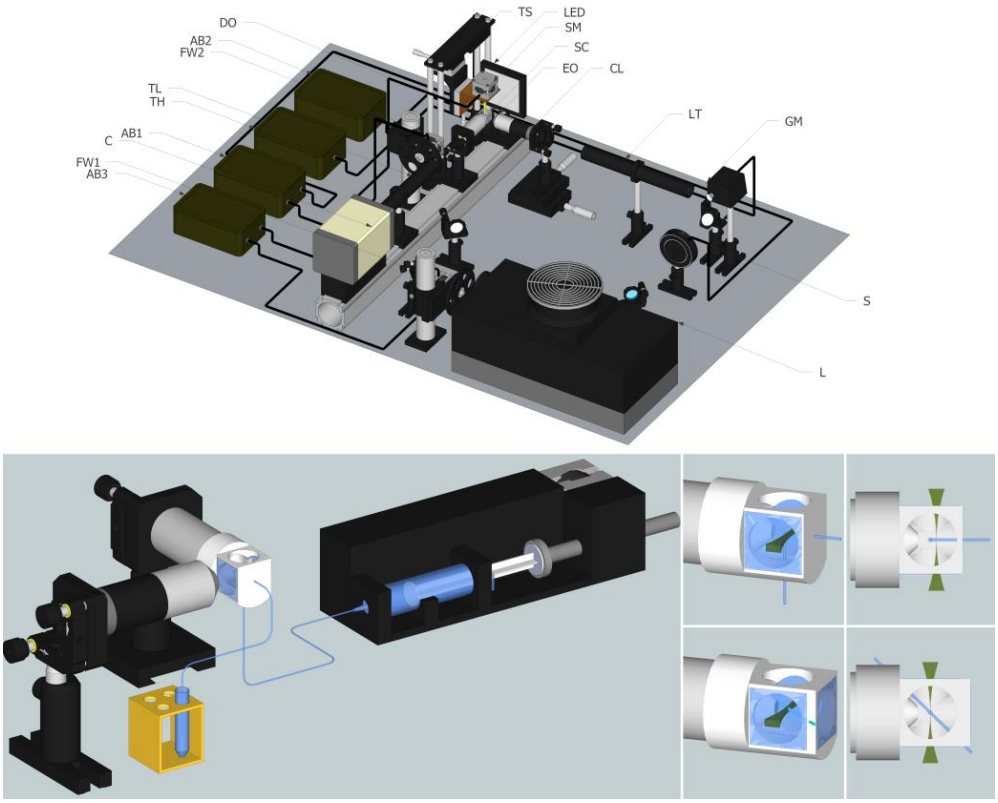

Fig. 1: Schematic of the Light Sheet Fluorescence Microscope at IGC (top). Detail of the SPIM-Fluid set-up (bottom).

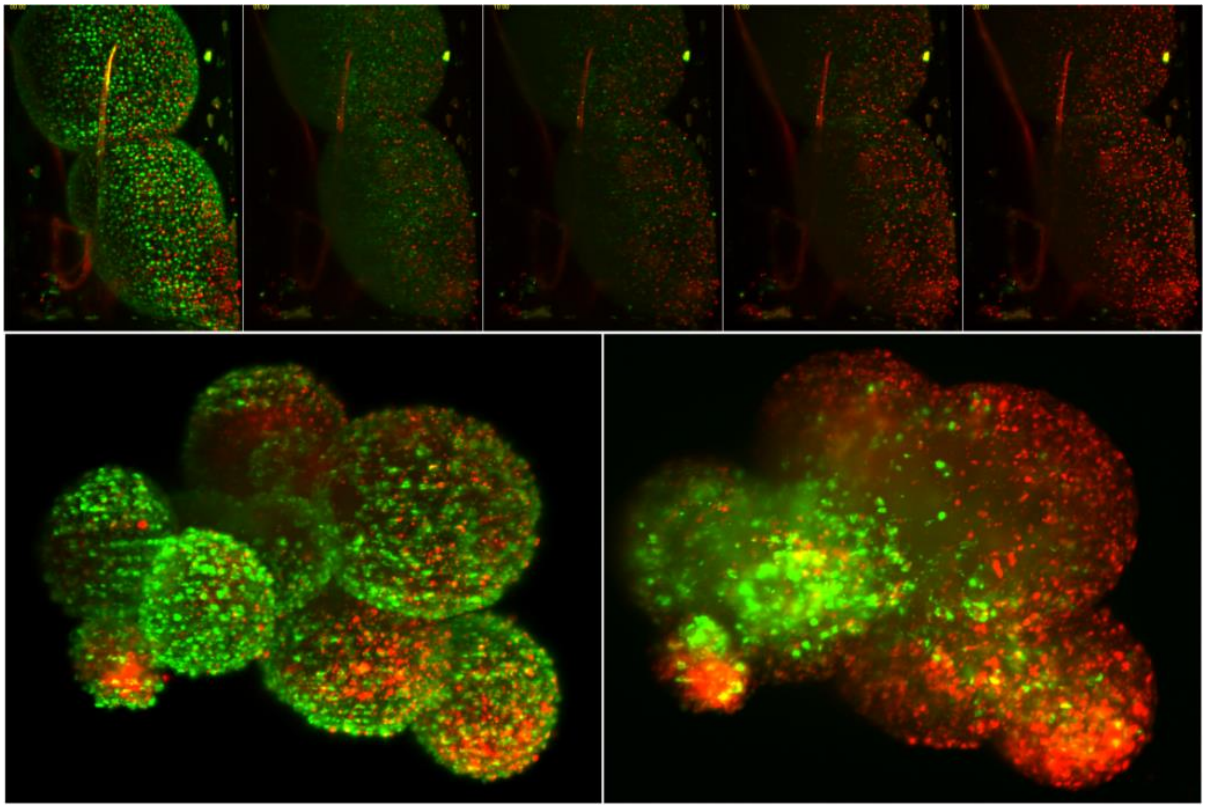

Fig. 2: Viability of cells within differentiated neurospheres visualized with NucView 488 and MitoView 633 Apoptosis Kit (Biotium, Hayward, CA, USA) image during 15 hours. Tert-butyl hydroproxide (tBHP) (Sigma), an oxidative stress inducer, was used to trigger apoptosis at a concentration of 1mM in Hibernate medium (Invitrogen).