

Congratulations to New ICFO PhD Graduate

Dr Matteo Bernardello graduated with a thesis entitled i¿½Development of novel multimodal light-sheet fluorescen e microscopes for in-vivo imaging of vertebrate organismsi¿½.

June 14, 2022

We congratulate Dr Matteo Bernardello who defended his thesis today in ICFO's auditorium. Dr Bernardello obtained his MSc degree in Telecommunications Engineering from the Universita degli Studi di Padova. He joined the Super-resolution Light Microscopy & Nanoscopy Laboratory at ICFO led by Dr Pablo Loza as a PhD student. Dr Bernardello's thesis entitled i¿½Development of novel multimodal light-sheet fluorescence microscopes f r in-vivo imaging of vertebrate organismsi¿½ was supervised by Dr Pablo Loza and Dr Emili J. Gua

ABSTRACT:

The observation of biological processes in their native environments is of critical importance

for life science. While substantial information can be derived from the examination of in-vitro biological samples, in-vivo studies are necessary to reveal the complexity of the dynamics happening in real-time within a living organism. Between the possible biological model choices, vertebrates represent an important family due to the various characteristics they share with the human organism. The development of an embryo, the effect of a drug, the interaction between the immune system and pathogens, and the cellular machinery activities are all examples of highly-relevant applications requiring in-vivo observations on broadly used vertebrate models such as the zebrafish and the mouse.

To perform such observations, appropriate devices have been devised. Fluorescence microscopy is one of the main approaches through which specific sample structures can be detected and registered in high-contrast images. Through micro-injections or transgenic lines, a living specimen can express fluorescence and can be imaged through such microscopes. Various fluorescence microscopy techniques have been developed, such as Widefield Microscopy (WM) and Laser Scanning Confocal Microscopy (LSCM). In WM the entire sample is visualized in a single 2D image, therefore losing the depth information, while LSCM can recover the 3D information of the sample but with inherent limitations, such as phototoxicity and limited imaging speed.

In the last two decades, Light-Sheet Fluorescence Microscopy (LSFM) emerged as a technique providing fast and 3D imaging, while minimizing collateral damages to the specimen. However, due to the particular configuration of the microscope's components, LSFM setups are normally optimized for a single application. Also, sample management is not trivial, as controlling the specimen positioning and keeping it alive for a long time within the microscope needs dedicated environmental conditioning.

In this thesis, I aimed at advancing the imaging flexibility of LSFM, with particular attention to sample management. The conjugation of these aspects enabled novel observations and applications on living vertebrate samples.

In Chapter 1, a brief review of the concepts employed within this thesis is presented, also pointing to the main challenges that the thesis aims to solve.

In Chapter 2, a new design for multimodal LSFM is presented, which enables performing different experiments with the same instrument. Particularly, high-throughput studies would benefit from this imaging paradigm, conjugating the need for fast and reproducible mounting of multiple samples with the opportunity to image them in 3D. Additionally, from this design, a transportable setup has also been implemented.

With these systems, I studied the dynamics of the yolk's microtubule network of zebrafish embryos, describing novel features and underlining the importance of live imaging to have a whole view of the sample's peculiarities. This is described in Chapter 3.

Further applications on challenging live samples have been implemented, monitoring the macrophage recruitment in zebrafish and the development of mouse embryos. For these applications, described in Chapter 4, I devised specific mounting protocols for the samples,

keeping them alive during the imaging sessions.
In Chapter 5, an additional LSFM system is described, which allows for recording the sub-cellular machinery in a living vertebrate sample, while avoiding its damage thanks to the devised sample mounting. Through this, single-molecule microscopy (SMM) studies, normally performed on cultured cells, can be extended to the nuclei of living zebrafish embryos, which better recapitulate the native environment where biological processes take place.
Finally, Chapter 6 recapitulates the conclusions, the impacts, future integrations, and experimental procedures that would be enabled by the work resumed in this thesis.

Thesis Committee:
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