

PhD THESIS DEFENSE: Development of novel multimodal light-sheet fluorescence microscopes for in-vivo imaging of vertebrate organisms

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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, als pointing to the main challenges that the thesis aims to solve

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

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

Further applications on challenging live samples have been implemented, monitoring th macrophage recruitment in zebrafish and the development of mouse embryos. For thes 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 th sub-cellular machinery in a living vertebrate sample, while avoiding its damage thanks to th devised sample mounting. Through this, single-molecule microscopy (SMM) studies normally performed on cultured cells, can be extended to the nuclei of living zebrafis embryos, which better recapitulate the native environment where biological processes tak place

Finally, Chapter 6 recapitulates the conclusions, the impacts, future integrations, an experimental procedures that would be enabled by the work resumed in this thesis

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