USE OF LIGHTSHEET IMAGING TO ASSESS PHARMACOLOGICAL MANIPULATION EFFECTS ON ZEBRAFISH NEURAL DEVELOPMENT

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The nervous system is responsible for the coordination of vital activities and behaviors. An enduring challenge in neural development studies is to decipher and describe how neurons are generated, differentiate and connect to create highly sophisticated functional neural networks [1]. An established approach to study these processes is to pharmacologically manipulate the neural development of model organisms and analyze the effects through fluorescence microscopy. However, none of these studies were performed *in vivo* for periods of at least 24 hours, due to the challenges of imaging live specimens with high time resolution over long periods [2]. Therefore, these inherently highly dynamic neuronal processes occurring over long-term periods are still poorly understood.

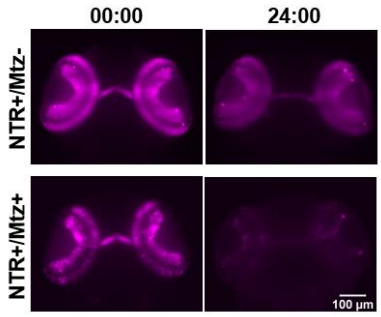

Figure 1: Treated/untreated zebrafish retina – time 0h and 24h.

In this project, we explore the potential of lightsheet microscopy to observe *in vivo* the effects of pharmacological manipulation on the neural development of zebrafish at the cellular level. As a proof of concept, we employed the chemical-genetic method of nitroreductase/metrodinazole-mediated ablation [3] of retinal cells in 3 days post fertilization zebrafish. We show for the first time that it is possible to image the ablation effects with high temporal resolution (every 5 minutes) for extended periods of time (up to 24 hours). This technique may potentially answer unaddressed questions in the neuroscience field, from the structure and function of subpopulations of neurons to their role in animal behavior.

In addition, we developed an innovative imaging chamber for drug testing in long term *in vivo* imaging. The chamber is optically transparent, biocompatible and of small inner volume (~3 mL), which increases the efficiency of drug-based experiments by reducing experimental costs and production of residues. Using the new chamber, we successfully replicated the ablation results previously obtained without it. The application of the chamber is not limited to neuroscience studies but could also be employed in other scientific fields that address pharmacological manipulation in living specimens.

Figure 2: Developed imaging chamber.

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