Application note

Fast functional imaging of the developing visual system of zebrafish

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Zebrafish is a valuable model for studying the vertebrate visual system. The transparency of zebrafish during its early development allows the use of fluorescent microscopy. We investigated the optic tectum's functional development by imaging neural activity from three to five days post-fertilization (dpf), when the zebrafish visual system rapidly develops. We performed fast functional live cell imaging of neural activity using a genetically encoded calcium indicator expressed in the central nervous system of zebrafish larvae. Comparing the neural response to visual stimuli between fish exposed to and deprived of visual information during development, we evaluated the function impact of visual experience in the optic tectum.

Keywords: Live cell imaging, Confocal microscopy, Zebrafish, Neural activity, Optic tectum

INTRODUCTION

Larval zebrafish (*Danio rerio*) is a widely used model organism in neurobiology. In particular, zebrafish has emerged as a popular model for the study of the vertebrate visual system. Although the zebrafish visual system has been rigorously studied for over two decades, early on research was focused on characterizing its anatomical and developmental organization (Stuermer, 1988; Easter, Jr. and Nicola, 1996). With the developments of fluorescent microscopy and relevant labels, it is now possible to perform whole brain functional as well as high resolution structural imaging. Confocal microscopy provides high-resolution imaging of neuron morphology and synaptic connections in fine detail, yet it comes with several caveats, especially during imaging in vivo. Firstly, the confocal microscope used must be able to image deep into the tissue with minimal light scattering. Secondly, to obtain the least invasive imaging, a confocal microscope must exhibit minimal phototoxicity. In addition, imaging is to be performed with high speeds to allow good temporal resolution of a neural response. All three criteria listed above are fulfilled with the NL5+ Line *RE*scan confocal system.

NEURAL RESPONSE TO VISUAL STIMULI

In this application note, we will focus on the functional imaging of the optic tectum (OT). Located dorsally, OT is the largest visual processing area in the zebrafish brain (Fig. 1). Here, visual and somatosensory information are combined to regulate behavioural motor responses making it a key target to understand the functional development of the visual system (Bianco and Engert, 2015; Helmbrecht et al., 2018).

To measure the functional response of the developing visual system to visual stimuli, fish were exposed to a bright light during imaging sessions. Using zebrafish expressing the genetically encoded calcium indicator GCaMP6, calcium dynamics in response to the stimuli were measured throughout the optic tectum. Using two transgenic backgrounds expressing GCaMP6 under different promotors, both pre-and post-synaptic dynamics at the retinotectal synapse could be assessed.

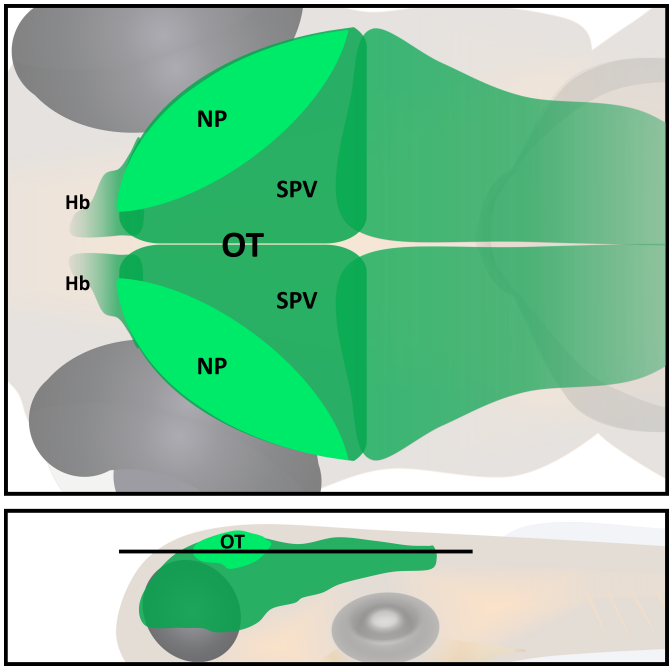

Figure 1. Schematic overview of the larval zebrafish brain at 5 days post fertilization. OT – Optic Tectum (sensory information processing center, contains SPV and NP), SPV – Stratum Periventriculare (cell bodies of most tectal neurons), NP – Neuropil (retinal ganglion cell axon terminals), Hb – Habenulae (part of the dorsal-diencephalic conduction system that interconnects sites in the limbic forebrain with the ventral midbrain and hindbrain). The visual system is imaged dorsally in the horizontal plane (black line).

Several imaging speeds were tested, ranging from 3 to 50 frames per second (fps or Hz) over the full field of view of 2k x 2k pixels using a camera with the pixel size of 6.5 µm. The speed of 10 Hz was selected as it was estimated to be the sweet spot for both the

spatial and temporal resolution in our experiments. As shown in Fig. 2, utilizing this imaging speed of the NL5+ Line *RE*scan system, detailed time series were acquired. By limiting the laser power, imaging was performed on the live samples for an extended amount of time with limited photobleaching. As a result, the detailed whole brain time series was analysed to identify calcium spikes on a pixel-by-pixel basis.

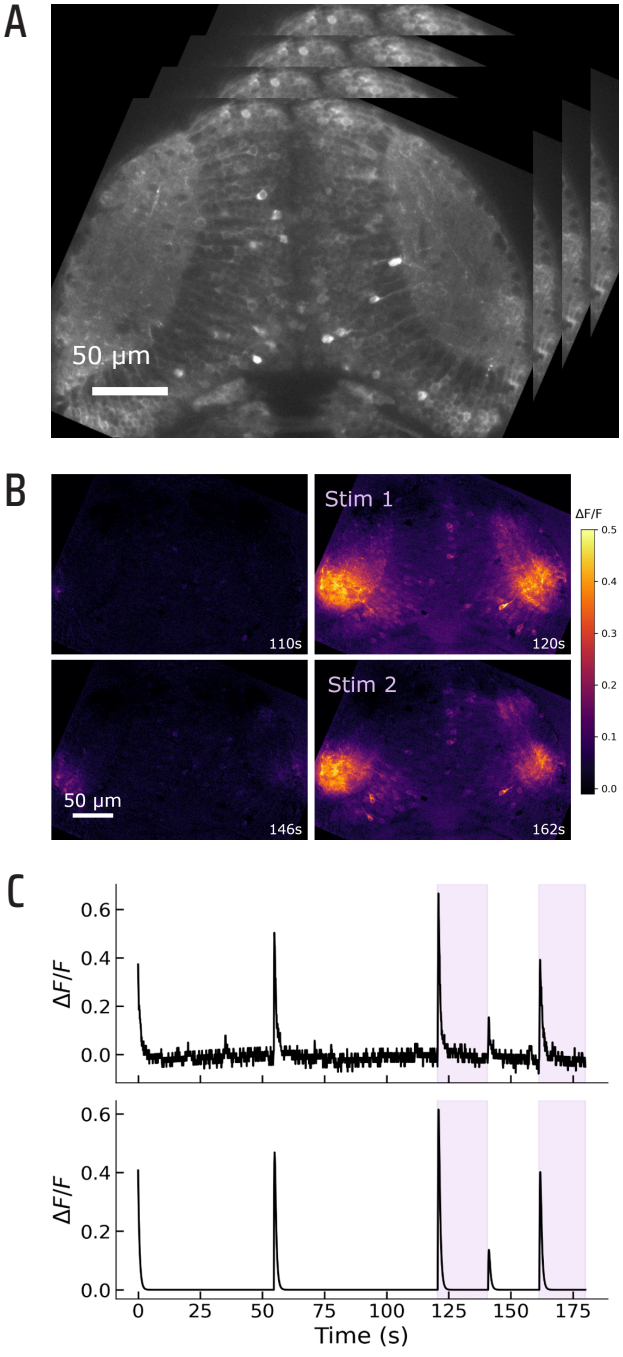

Figure 2. Image analysis workflow for imaging neural response to a visual stimulus. **A)** During imaging a timeseries of individual images were collected at 10 Hz using a 30xSil/1.05NA objective. The resulting field of view allows for the detection of calcium dynamics throughout the brain of larval zebrafish. **B)** After pre-processing the signal-to-baseline (ΔF/F) timelapses show calcium dynamics throughout the brain, allowing us to compare frames with (left) or without visual stimulation (right). **C)** By measuring the ΔF/F per pixel or region of interest, for example, single neurons, neural activity can be quantified by detecting the characteristic spikes in fluorescence.

NL5+ Line *RE*scan confocal microscope turned out ideal for the in vivo imaging of biological processes in model organisms such as the larval zebrafish as they are designed to image photogentle. During some of the imaging sessions, we performed extended time-lapse sessions of up to 6 hours of continuous imaging of live samples. In addition, using an objective with a long working distance (WD) allowed us to image more than half a millimetre deep into the sample with minimal light scattering.

CONCLUSION

Using the NL5+ Line *RE*scan confocal microscope, three, four and five days old zebrafish larvae were imaged to access their neural response to a visual stimulus. During this period the visual system is quickly developing, which leads to changes in neural dynamics in response to visual stimuli. Being able to observe these dynamics with a confocal microscope with such a large field of view is made possible by the fast, deep and gentle imaging that line rescanning provides.

NL5+ LINE *RE*SCAN CONFOCAL SYSTEM OVERVIEW

NL5+ Line *RE*scan is a versatile add-on confocal which can be combined with a number of different laser combiners, most sCMOS cameras and virtually any widefield microscope to create an advanced fast scanning confocal imaging system.

- Speed up to 50 fps at full FOV of FN18
- Compatible with Upright and Inverted Microscope
- 6 positions Emission Filter Wheel

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