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Two-photon high-speed light-sheet volumetric imaging of brain activity during sleep in zebrafish larvae

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Abstract

Although it is well known that zebrafish display the behavioural signature of sleep, the neuronal correlates of this state are not yet completely understood, due to the complexity of the measurements required. For example, when performed with visible excitation light, functional imaging can disrupt the day/night cycle due to the induced visual stimulation. To address this issue, we developed a custom-made two-photon light-sheet microscope optimized for high-speed volumetric imaging. By employing infra-red light (not visible to the larva) for excitation, we are able to record wholebrain neuronal activity with high temporal- and spatial-resolution without affecting the sleep state. In two-photon light-sheet microscopy the maximum achievable frame rate is limited by the signal-to-noise ratio. To maximize this parameter, we optimized our setup for high peak power of excitation light, while finely controlling its polarisation, and we implemented remote scanning of the focal plane to record without disturbing the sample. Using this setup, as a preliminary result, we characterized the intensity spectra of neuronal calcium traces of 4 days post fertilisation larvae during the day/night phases. We aim to extend these results to multiple brain regions and frequency bands.

Citation

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