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Two-photon imaging of neural population activity in zebrafish

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ABSTRACT

Rapidly developing imaging technologies including two-photon microscopy and genetically encoded calcium indicators have opened up new possibilities for recording neural population activity in awake, behaving animals. In the small, transparent zebrafish, it is even becoming possible to image the entire brain of a behaving animal with single-cell resolution, creating brain-wide functional maps. In this chapter, we comprehensively review past functional imaging studies in zebrafish, and the insights that they provide into the functional organization of neural circuits. We further offer a basic primer on state-of-the-art methods for in vivo calcium imaging in the zebrafish, including building a low-cost two-photon microscope and highlight possible challenges and technical considerations.

1. Introduction

1.1. Imaging neural population activity

Understanding how dynamic activity in networks of neurons allows us to process sensory stimuli, make decisions and execute appropriate actions is a central goal of neuroscience research. Over the last decades, much has been learned about the spiking properties of individual neurons and the macroscopic organization of brain areas, but we know relatively little about the intermediate scale: the spatiotemporal patterns of activity in interconnected populations of neurons in the brain of a behaving animal [1]. This can be attributed mostly to technical constraints: electrophysiology is limited to recording from at best a small subset of neurons, and is usually blind to their identity. Recently, fluorescence-based functional imaging methods have opened up the possibility to record simultaneously from hundreds, or even thousands of neurons, with very high time resolution [2]. Beyond a simple scaling up of existing recording methods, these imaging techniques also provide access to new kinds of information, such as the spatial structure of circuits at the cellular and even synaptic level [3] and the genetic identity of the recorded neurons. Both the imaging technology, and the methods for analyzing and understanding the very large data sets that can be acquired are still in a state of rapid development, and we will endeavor in this article to give an overview of both the current state of the art, and also the outlook for the future.

1.2. The zebrafish

The zebrafish model, which was originally developed because of its optical transparency at early developmental stages, is uniquely well suited to take advantage of advances in optical neurophysiology [4–8]. The whole brain of a five-day-old larva occupies a volume about 800 μm long, 400 μm wide and 300 μm thick and contains on the order of 100,000 neurons. Thus, using standard imaging equipment, one entire coronal section through the brain can be imaged with subcellular resolution in a single field of view (Fig. 1). Larval fish can be partially restrained using low-melting temperature agarose, allowing stable imaging of the brain, even while the eyes and tail are free to move, enabling the correlation of neural activity with behavior [9,10]. Moreover, efficient transgenic methods are available that allow straightforward expression of genetically encoded tools in defined neuronal populations [11].

1.3. Two-photon microscopy

We focus here on one particular imaging method: two-photon laser scanning microscopy [12]. Developed more than two decades ago by Denk and colleagues, this technique takes advantage of nonlinear photon absorption to confine fluorescence excitation to a small focal volume (Fig. 2B), which can then be scanned in a raster pattern through a sample to yield an image. This has a number of advantages for in vivo studies of neural circuits. Images can be obtained with excellent 3D resolution, with minimal out of focus fluorescence excitation and consequent photo-damage. The technique works very well in scattering tissue, because weakly scattered infrared light is used for excitation, and efficient, non image-forming methods can be used for collection of fluorescence

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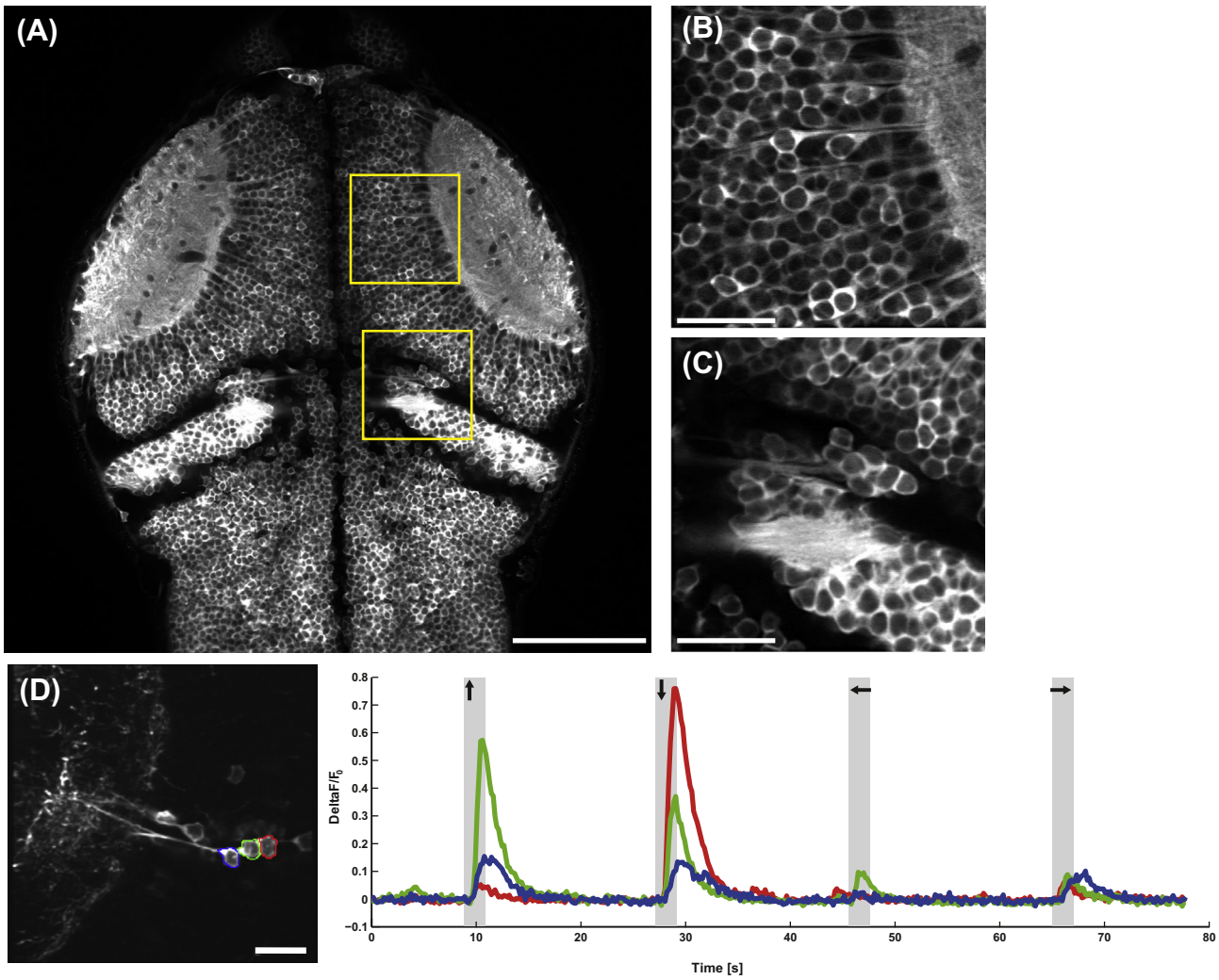

Fig. 1. Two-photon imaging in the zebrafish brain. (A) A two-photon optical section through the brain of a fish expressing a pan-neuronal fluorescent marker (HuC:YC2.1) [110]. Scale bar 100 μm. (B, C) Detail images showing subcellular resolution. Dark circles are nuclei of neurons in the tectum and cerebellum. Scale bar 25 μm. (D) Imaging of visually evoked, orientation and direction selective calcium signals in tectal neuron somata. Left: two-photon image showing tectal neurons transiently expressing the genetically encoded indicator GCaMP5G, following embryo injection with HuC:Gal4FF and UAS:GCaMP5G plasmids. Scale bar 20 μm. Right: delta *F*/*F* traces for three somatic regions of interest during presentation of dark bars that sweep across a screen below the fish over 2 s, in four different directions. First bar is in tail to head direction; average of 2 repeats.

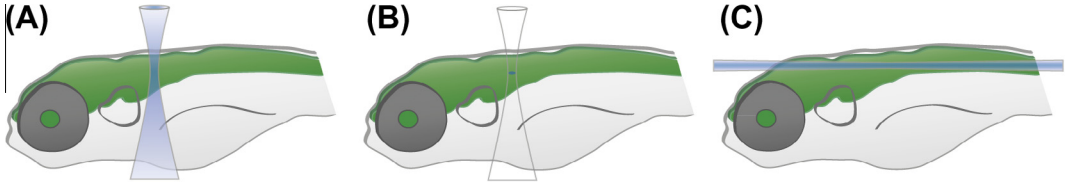

Fig. 2. Schematic of imaging volumes for three common methods of 3-dimensional fluorescence microscopy. (A) In a confocal, a laser beam is focused to a spot, but also excites fluorophores in planes above and below the point of focus. (B) In a two-photon microscope, an infrared laser beam passed through the sample, but fluorescence excitation is confined to the focal point. (C) In a light-sheet microscope, a thin beam or sheet of excitation light enters the specimen at right angles to the imaging path, so only fluorophores in the focal plane of the objective are excited.

emission. Importantly also, when imaging in tiny zebrafish larvae, the excitation light is mostly invisible, so should not affect their behavioral responses, particularly to visual stimuli. Fig. 2 shows a comparison of excitation volumes using three methods: confocal laser scanning, two-photon and light-sheet imaging (see Section 7). Current two-photon microscopy techniques allow imaging of neuronal activity in mammalian neocortex at depths of up to 1 mm [13], imaging from freely moving animals via optic fibers [14] and detection of single action potentials with near-millisecond precision [2].

1.4. Calcium imaging

Monitoring membrane potentials can provide an accurate measure of the timing and location of neuronal activity. Yet, most functional imaging studies do not aim to measure membrane voltage