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BonZeb: open-source, modular software tools for high-resolution zebrafish tracking and analysis

Nicholas C. Guilbeault[1,2], Jordan Guerguiev[1,2], Michael Martin[1,2], Isabelle Tate[1,2] & Tod R. Thiele[1,2]✉

We present BonZeb—a suite of modular Bonsai packages which allow high-resolution zebrafish tracking with dynamic visual feedback. Bonsai is an increasingly popular software platform that is accelerating the standardization of experimental protocols within the neurosciences due to its speed, flexibility, and minimal programming overhead. BonZeb can be implemented into novel and existing Bonsai workflows for online behavioral tracking and offline tracking with batch processing. We demonstrate that BonZeb can run a variety of experimental configurations used for gaining insights into the neural mechanisms of zebrafish behavior. BonZeb supports head-fixed closed-loop and free-swimming virtual open-loop assays as well as multi-animal tracking, optogenetic stimulation, and calcium imaging during behavior. The combined performance, ease of use and versatility of BonZeb opens new experimental avenues for researchers seeking high-resolution behavioral tracking of larval zebrafish.

The ability to precisely track animal movements is vital to the goal of relating the activity of the nervous system to behavior. The combination of precision tracking with systems for behavioral feedback stimulus delivery can provide valuable insights into the relationship between sensory inputs and behavioral outputs[1–3]. Methods for high-resolution behavioral tracking and dynamic sensory feedback can be combined with methods for monitoring or manipulating neural circuit activity to allow researchers to explore the neural circuitry underlying sensorimotor behaviors. These assays can also increase the throughput of behavioral experiments through the rapid and repeatable delivery of stimuli, and allow researchers to probe the sensorimotor loop by controlling and perturbing sensory feedback[4–6].

Despite its significance, the use of behavioral feedback technology is not standard across laboratories investigating sensorimotor integration. A key challenge in developing behavioral feedback systems is synchronizing high-throughput devices. Relatively few software implementations have succeeded in addressing this synchronization problem and most rely on software configurations that require advanced levels of technical expertise. Bonsai, an open-source visual programming language, has recently gained traction among the neuroscience community as a powerful programming language designed for the rapid acquisition and processing of multiple data streams[7]. Currently, there are no released Bonsai packages that allow for high-speed kinematic tracking of small model organisms, such as the larval zebrafish, while providing behavior based stimulus feedback. Here, we present BonZeb, an open-source, modular software package developed entirely in Bonsai for high-resolution zebrafish behavioral tracking with virtual open-loop and closed-loop visual stimulation.

Results

Overview. BonZeb provides an open-source and approachable method to implement the functionalities of extant behavioral feedback systems. Furthermore, BonZeb supplies a range of novel utilities that extend Bonsai's capabilities (Fig. 1). We developed packages for high-speed video acquisition, presentation of a visual stimulus library, high-resolution behavioral tracking, and analysis. BonZeb inherits Bonsai's reactive programming framework for processing synchronous and asynchronous data streams (Fig. 2A). The reactive framework allows users to process incoming streams of data, regardless of whether the data are coming from a finite source (*e.g.* a saved video) or continuous source (*e.g.* streaming from a camera). There are four major classifications of nodes—source nodes, transform nodes, sink nodes, and combinators—which generate or manipulate streams of data called observable sequences (Fig. 2B). Figure 2B also provides a basic example of how BonZeb performs online behavioral tracking. An online manual for BonZeb provides details for running the presented packages

[1]Department of Biological Sciences, University of Toronto Scarborough, Toronto, Canada. [2]Department of Cell and Systems Biology, University of Toronto, Toronto, Canada. ✉email: tod.thiele@utoronto.ca

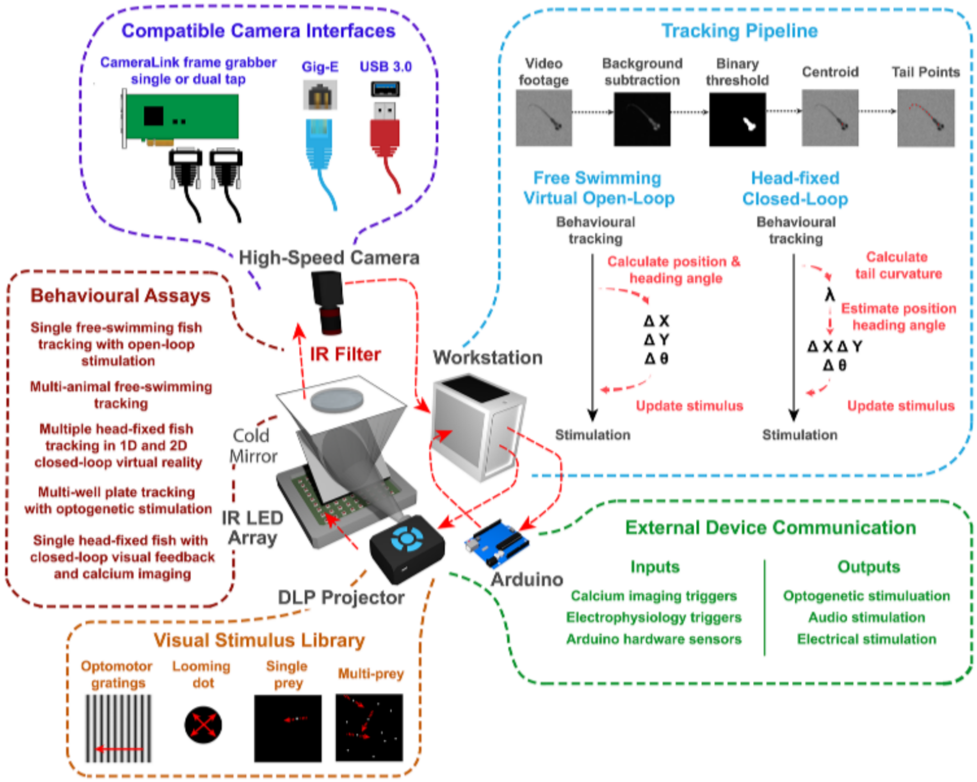

Figure 1. Overview of the behavioral feedback system. The behavioral feedback hardware consists of modular components based on a previous design[5]. High-speed cameras convey live video of behaving larval zebrafish to a workstation, which tracks the behavior of the fish and generates output signals to an external device to provide sensory stimulation. The workstation processes incoming video frames with BonZeb's customizable tracking pipeline. This pipeline transforms behavioral data into virtual open-loop stimulation for free-swimming fish or closed-loop stimulation for head-fixed fish. BonZeb can interface with Arduino boards, display devices, data acquisition boards, etc., for receiving or sending data. BonZeb also includes a library of common visual stimuli for closed-loop and open-loop visual stimulation. Components of the behavioral feedback system are not to scale.

as well as a knowledge base for the development of new complex tracking assays (https://github.com/ncguilbeault/BonZeb).

Free-swimming behavior in virtual open-loop. BonZeb can visually evoke and capture the core behavioral repertoire of freely-swimming larval zebrafish using virtual open-loop assays[4]. To evoke predator avoidance visual escapes, larval zebrafish were presented from below with an exponentially expanding looming dot stimulus to either the left or right visual field at a 90° angle relative to the heading direction (Fig. 3A). Similar to a previous study[8], we found that fish stimulated with a looming dot in virtual open-loop produced escape responses characterized by a large initial turn away from the stimulus followed by high-frequency tail oscillations (Figs. 3A, 4A, Supplemental Video 1, Supplemental Video 2—700 Hz acquisition). These escape responses were easily identified from other non-escape responses, as the max initial heading angle was consistently greater than 50° and the max velocity exceeded 15 cm/s in all elicited escape responses (Fig. 4A, bottom left). The max bending angle of the initial turn of the escape depended on the location of the looming stimulus such that the initial turn consistently oriented the fish away from the stimulus (left loom escapes, $n = 5$, $M = 100.7$, $SD = 27.3$, right loom escapes, $n = 6$, $M = -115.3$, $SD = 33.4$, two-tailed t-test: $t_{10} = 10.47$, $p < 0.001$; Fig. 4A, bottom right).

Fish were stimulated to produce the optomotor response (OMR) under virtual open-loop conditions by continually updating the orientation of a drifting black and white grating. In our open-loop OMR assay, fish were presented with an OMR stimulus from below that was tuned to always drift in a constant direction relative to the heading angle (90° left or right). Consistent with previous studies[5,9], we observed that fish continually produced turns and swims to follow the direction of optic flow (Fig. 3B, Supplemental Video 3). Fish produced significantly more clockwise turns when stimulated with rightward OMR ($n = 10$, $M = 3.8$, $SD = 2.5$) compared to when stimulated with leftward OMR ($n = 10$, $M = -4.0$, $SD = 1.6$, two-tailed t-test: $t_{19} = 7.85$, $p < 0.001$, Fig. 4B).

We also developed a novel virtual hunting assay where a small white spot is presented from below. Figure 3C shows an example of a behavioral sequence from a fish stimulated with a virtual dot moving back and forth along an arc of 120° positioned 5 mm away from the fish. This fish produced two J-turns when the stimulus was in a lateral position followed by two approach swims as the stimulus moved toward the frontal field. In this example,

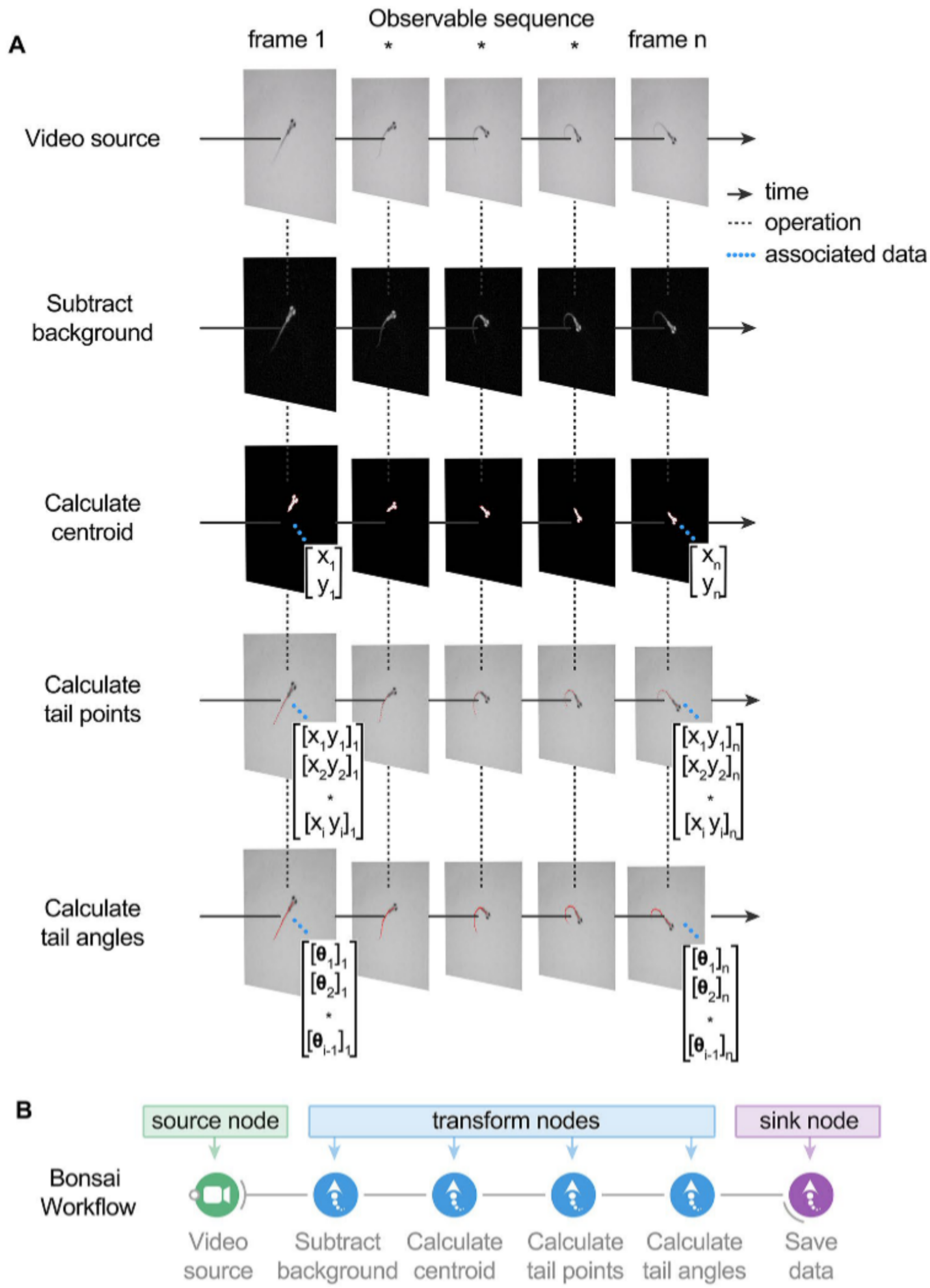

Figure 2. BonZeb inherits Bonsai's reactive architecture for processing data streams. (**A**) A video source node generates images over time. The video source can either be a continuous stream of images from an online camera device or a previously acquired video with a fixed number of frames. A series of transformation nodes are then applied to the original source sequence. Each transformation node performs an operation on the upstream observable sequence that is then passed to downstream nodes. A typical pipeline consists of background subtraction, centroid calculation, tail point calculation, and finally, tail angle calculation. Nodes have a unique set of visualizers that provide the node's output at each step. Each node has a set of properties associated with the output, such as a single coordinate, an array of coordinates, or an array of angles, which can be used for more sophisticated pipelines. (**B**) Bonsai workflow implementation of the above data processing pipeline. An additional node is attached at the end of the workflow to save the tail angles data to a csv file on disk. There are 4 different general classifications of nodes in Bonsai. Source nodes (green) generate new observable sequences and do not require inputs from upstream nodes. Transform nodes (blue) perform an operation on the elements of an upstream observable sequence to produce a new observable sequence that can be subscribed to by downstream nodes. Sink nodes (purple) perform side operations with the elements of the data stream, such as saving data to disk or triggering an external device. Sink nodes then pass along the upstream observable sequence to subscribed downstream nodes without modifying any of the elements of the upstream observable sequence. Combinator nodes (not shown here) are important for combining sequences and become crucial for more complex pipelines (see online manual for examples).