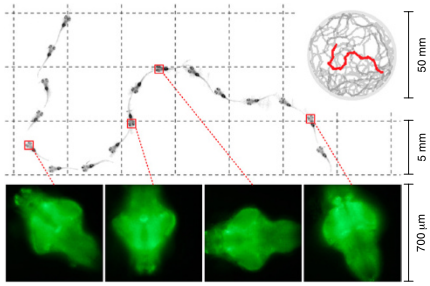
A DIFFerent way to keep up with zebrafish

Building a neurological model of the brain is a complex task. It requires gathering data on thousands to billions of neurons in an effort to understand the networks and how they interact. Zebrafish larvae are a popular animal model in neuroscience because their transparent skin allows scientists to use microscopes to view fluorescent signals of neural activity at cellular resolution, which in turn can be used to produce network maps in response to behaviors or stimuli.

But there's a catch: to build a realistic and functional map of brain networks, scientists must capture data during natural behaviors such as navigation, feeding, or social interactions, all of which require freedom of movement. Larval tzebrafish dart around much faster than current imaging systems can follow. "The acceleration is the same as a Formula One racecar. It's faster than anything people have tried to chase down with single cell resolution microscopy," says Drew Robson, a fellow at the Rowland Institute at Harvard.

Robson and co-investigator Jennifer Li, while working on their PhD theses, were frustrated because existing technology couldn't track the larval fish quickly enough. To adequately image brain activity, the zebrafish larvae had to be immobilized—either by paralysis, or by immersing them in gelatinous agar. "In terms of thinking about what the brain does in ordinary life, this is the worst possible situation to put the animal in. It can't express the natural behaviors that make the brain so complicated and interesting in the first place," said Robson.

And that's unfortunate, because these proto-fish are surprisingly complex. They hunt microorganisms using different strategies, and they even exhibit introverted and extroverted social behavior. "All of that was difficult to study," says Li, who is a fellow at the Rowland Institute.



Top, a sample trajectory of a freely swimming larval zebrafish in a 16-s interval. This trajectory is highlighted (inset; red line) among all movements (inset; gray lines) in the behavior arena in an 8-min interval. Bottom, examples of fluorescence images of the brain obtained during this sample trajectory. Adapted from *Nat. Methods*, doi:10.1038/nmeth.4429, published online 11 September 2017.

The solution was to jettison the agar and allow the animals to swim freely, but that would require powerful software that could predict movements and thus cancel brain movements. The researchers turned to engineering for a solution in the form of optimal control theory. The software involved uses mathematical models that predict the future position of the brain, and calculates the best path for the motorized staging system to successfully follow it. The technique has rarely seen use in biology—instead, it is typically reserved for mission critical functions such as operational control in chemical manufacturing plants, or drone guidance systems. "Any time ordinary control is not fast or accurate enough," says Robson.

Li and Robson combined brain tracking with a variant of HiLo microscopy to build a system they call Differential Illumination Focal Filtering (DIFF) microscopy (*Nat. Methods*, doi:10.1038/nmeth.4429; published online 11 September 2017). The method can image 100 optical sections per second, encompassing two brain volumes per second. It constantly tracked a free swimming larval zebrafish for one hour. Over that time, a majority of the specimen's brain was in the field of view more than 98% of the time.

The behavior arena was 50 mm in diameter and 750 µm deep, which is greater than 12 times the animal's body length. In a demonstration, Li and Robson applied four thermal gradients to the arena, and produced brain-wide neuron maps that might reflect the brain activity involved in determining turning angle and turn speed, as well as the response to absolute and relative temperatures. A large group of neurons were activated in response to both absolute temperature and turn speed, suggesting a link between sensory perception and behavioral strategy.

The findings affirm the technique's ability to map complex behaviors. Turn angle, turn speed, and temperature sensitivity represent the internal logic occurring in the brain. "It's all of the things you would need to have a coherent strategy rather than just flailing left and right," says Robson.

Going forward, the researchers hope to tweak the system to further increase the amount of time that an animal can be viewed.

"We could watch the animal grow, develop, and learn new things over days and maybe weeks. We could get all sorts of really cool information, about how animals make decisions, and what is means to be hungry, tired, or content," says Li.

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