Reading Minds with Lasers: Extracting the Neural Activity of *C. elegans*

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By using light-sheet microscopy and transgenic expression of fluorescent marker tags such as mCherry (a red fluorescent protein) and GCaMP6s (a calcium-sensitive green fluorophore)[1], the position and neuronal activity of a majority of neurons in the head of *C. elegans* can be simultaneously imaged non-invasively, in parallel, and *in vivo*. Under these experimental conditions, *C. elegans* can then be exposed to a range of anesthetics, creating an extraordinarily powerful experimental system for determining the effect of these agents on neuron-to-neuron communication. However, obtaining these 3D volumetric movies of the microscopic activity of the *C. elegans* nervous system is only the beginning of the process: the imaging data so produced may occupy several hundred gigabytes. The ability to track and to automatically extract neuronal activity from these large datasets and, in effect, to distill the data into a tractable form requires considerable algorithmic sophistication and access to supercomputing resources. This abstract describes how that technical process can be achieved, and consequently how the mind of a worm can be read.

Using a Dual Inverted Selective Plane Illumination (diSPIM) microscope (Applied Scientific Instrumentation, Eugene, OR), imaging was performed with a water-immersed 0.8 NA 40x objective (Nikon USA, Melville, NY) with illumination interleaved between a 488 nm laser at 5 mW power to excite GCaMP6s in the green and a 561 nm laser at 5 mW power to excite mCherry in the red (Vortran Laser Technology, Sacramento, CA). *C. elegans* neurons typically display slow transient changes in activity rather than rapid action potentials[2,3] and a volume acquisition rate of 2 volumes/second is sufficient to capture neuron dynamics while limiting photobleaching of the fluorescent markers. The images shown are two-dimensional representations of the underlying volumetric dataset at particular timesteps, with pixel intensities assigned using the Maximum Intensity Projection (MIP) ray-casting algorithm[4] as is commonly performed for visualizing PET and SPECT images.

The nuclei are first localized in the red channel via the nuclear expression of RFP. A set of implicit axes is created with the origin at the centroid of the data, with orthogonal axes generated using Principal Component Analysis from the second-order moments of the data. These axes will automatically move and turn to compensate for translation or rotation of the head of the worm, which helps to stabilize the tracking. The centerpoints of each nucleus are identified by convolving the red channel data with a Laplacian-of-Gaussian function[5] (colloquially known as a Sombrero function, due to its shape), which picks out nuclei by identifying bright spheres of a critical radius[6] such that 150 neurons in the head are tracked. The matching of neurons from one timestep to another was performed using the Kuhn-Munkres Assignment algorithm[7], always selecting the match that best minimizes the total squared-distance between neuron positions. All possible timestep matches are considered, not just proceeding forwards in temporal order. The identified locations of the nuclei are error-corrected using smoothing and consensus based on the location of the surrounding neurons[8]. Ultimately, a single complete chain of locations is established from the beginning to the end of the image sequence for each of the initially identified nuclei. The neuron activity is then extracted by averaging the green GCaMP signal in the soma of the neuron in a corona around the chain of 3D locations. This matching was performed in a massively parallel computation (Massachusetts Green High Performance Computing Center, Holyoke, MA). The tracking and extraction algorithms are written in Python, with inner-loop optimization in C.

There is obvious organization in the patterns of neural excitation in *C. elegans*. Our ongoing work is to determine how anesthetic agents disrupt these patterns in inter-neuronal communication.

Image:
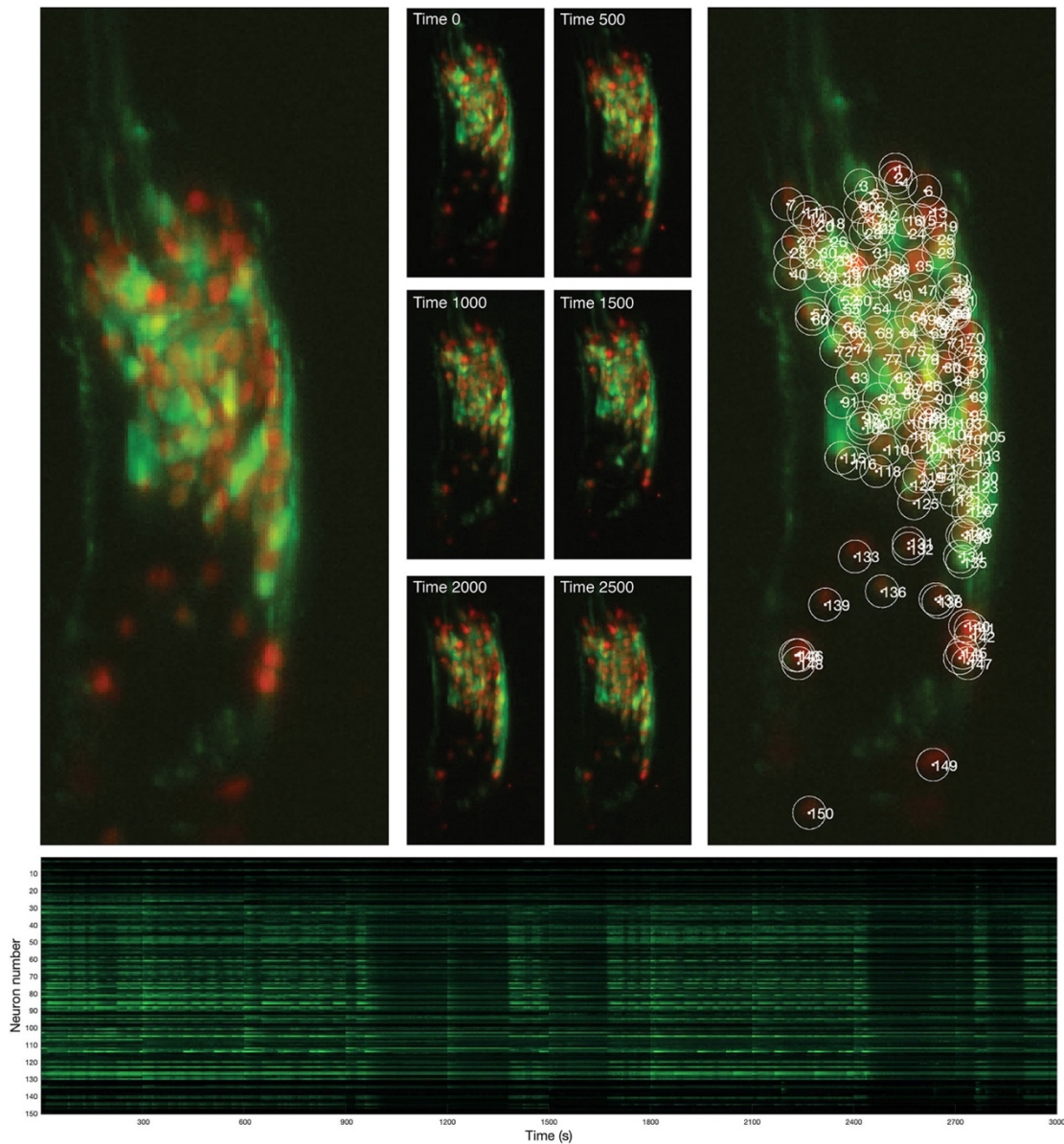
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