Computer Automated Neuron Identification in Functional Microscopy for *C. elegans*

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Current neuroimaging techniques are capable of capturing real-time neuronal activities, indicating areas of neuronal stimulation. However, state-of-art microscopes have compromised resolution, capturing the average neuronal activities of thousands of neurons in a single voxel. Due to this resolution limitation, the activities and interactions between individual neurons can only be inferred. To overcome the limitation, we have chosen hermaphrodite C. elegans with GCaMP and NeuroPAL transgene as our specimen. Hermaphrodite C. elegans' neuronal system consists of stereotyped 302 neurons. The GCaMP transgene allows for fluorescence in response to calcium levels, which serves as a proxy of neuronal activity. Calcium levels alone are insufficient to map individual neuronal activity because each neuron is susceptible to positional changes. To accurately locate individual neurons, neuroPAL transgene is implemented. NeuroPAL transgene is designed to characterize neurons with a unique fluorescence color, providing a means to differentiate neurons by its fluorescence coupled with approximate location. To accurately capture the positional and fluorescence data, images are acquired with Dual Inverted Selective Plane Illumination Microscope (DiSPIM). DiSPIM microscope has isotropic resolution, compared to the conventional confocal microscope which has compromised z-direction resolution. The acquired images are then processed with an automatic neuron labeling algorithm that labels each neuron with the be both position and color. Altogether, this algorithm allows tracing neuronal activity of individual neurons in real-time, which can be utilized to examine the effect of various stimuli on neuronal activity and interactions in a single neuron resolution.