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Whole-brain calcium imaging with cellular resolution in freely behaving Caenorhabditis elegans JEFFREY NGUYEN, FREDERICK SHIPLEY, ASHLEY LINDER, GEORGE PLUMMER, MOCHI LIU, SAGAR SETRU, JOSHUA SHAEVITZ, ANDREW LEIFER, Princeton University — The ability to acquire large-scale recordings of neuronal activity in awake and unrestrained animals is needed to provide new insights into how populations of neurons generate animal behavior. Acquiring this data, however, is challenging because it is difficult to track and image individual neurons as an animal deforms its posture and moves many body lengths. Here, we present an instrument capable of recording intracellular calcium transients from the majority of neurons in the head of a freely behaving *Caenorhabditis elegans* with cellular resolution while simultaneously recording the animals position, posture, and locomotion. 3D volumetric fluorescent images of neurons expressing the calcium indicator GCaMP6s are recorded at 6 head-volumes/s using spinning disk confocal microscopy. At the same time, we record low magnification images of the animal to measure the animals behavior and track its head as it moves. We develop a time independent neuronal matching algorithm that uses non-rigid point set registration and machine learning to correctly match neurons across time. Using this method, we are able to observe calcium transients from up to 90 neurons for over 4 min and correlate the neural activity with the animals behavior.

Jeffrey Nguyen
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