# SYNAPSE SEGMENTATION AND ANALYSIS REVEALS UNEXPECTEDLY BROAD SYNAPTIC REDISTRIBUTION IN NEONATAL BRAINSTEM

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Anatomical analyses of neurons and neural circuits commonly require segmentation of synaptic signal from noisy fluorescence images, a task for which automated methods are especially use. This can be especially challenging in studies of developing brain areas for which genetic tools are lacking, and/or in which large datasets are impractical. We individually labeled neurons of different ages in tissue from an immature auditory brainstem nucleus, immunostained for synaptic proteins, imaged at the confocal microscope, and reconstructed the labeled neurons together with their synaptic inputs. Although some existing software is capable of reconstructing the neuronal label accurately, we were unable to find an existing solution that was able to segment synapses accurately and efficiently for this dataset. As global thresholding algorithms are subject to error in this context, local thresholding algorithms have been developed to segment synapses in 3D [1]. However, such approaches can be unwieldy, requiring intensive user interaction and/or significant computational costs, whereas machine learning approaches can require datasets of impractical size. A further task is to determine whether two synaptic proteins are apposed to each other across synapses, a task for which correlation based co-localization methods fail to provide proximity information.

We will describe an algorithm for calculating and applying local threshold values to segment synapses while observing constraints on synapse size, and to identify proximate synaptic clusters. Our algorithm successfully segments synaptic clusters with nearby peaks, identifies synaptic partners within the same synapse, and is robust to noisy (unfiltered) datasets, with significant reduction in computational time. Applying our algorithm to datasets from immature auditory brainstem we make two unexpected findings: 1) excitatory synaptic terminals cluster in large numbers around the soma at early ages, and are redistributed away from the soma with age, and 2) inhibitory synapses are redistributed toward the soma, in the absence of auditory activity.

## REFERENCES

[1] J.L. Morgan; T. Schubert, and R.O.L. Wong, “Developmental patterning of glutamatergic synapses onto retinal ganglion cells,” *Neural Development*, **3**, 8 (2008).