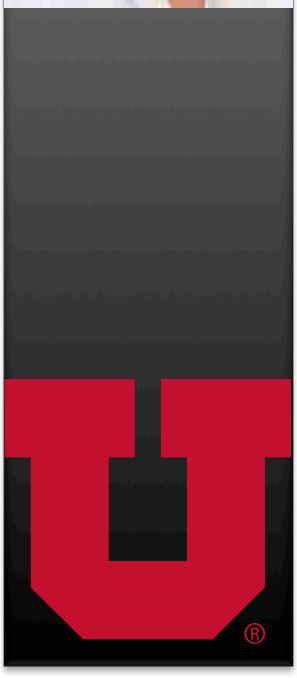

DEVELOPING METHODS FOR *IN VIVO* SUPER RESOLUTION MICROSCOPY

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Standard fluorescent microscopy techniques are limited by the diffraction of light and cell structures smaller than the diffraction of light cannot be resolved. Super resolution microscopy solves this problem and makes resolving structures smaller than the diffraction limit of light possible. However, background labeling of unwanted structures is pervasive with current labeling and fixation techniques and decreases the accuracy of images obtained. We aimed to improve these labeling and fixation techniques while also developing a method to simultaneously localize two proteins *in vivo* using the model organism *Caenorhabditis elegans*. Synaptotagmin-1 and RIM are two proteins involved in synaptic vesicle fusion and are partially *colocalized* within the nervous system. A strain of *C. elegans* expressing the two tagged proteins, synaptotagmin-1::snapf and RIM::halotag, was constructed. We compared fixed and live labeling techniques to determine the best method for super resolution imaging of these proteins. With a fixed labeling technique, we found that we could not sufficiently reduce the background levels for super resolution imaging. However, using cell permeable fluorophores conjugated to HALO or SNAPf ligands *in vivo,* we were able to successfully label synaptic structures within the nervous system in living animals. By further modifying the dye type, recovery time, recovery temperature, and fix type in live labeling and fixation techniques we were able to decrease the amount of background labeling and perform super resolution imaging of the nervous system in an intact animal. Through these experiments labeling and fixation techniques were improved to optimize super resolution imaging and we now possess a way to image two proteins simultaneously and accurately in *C. elegans*. These techniques were tested on specific synaptic proteins but can be generalized to all proteins and provide a more accurate way to study protein localization and interactions using super resolution microscopy.

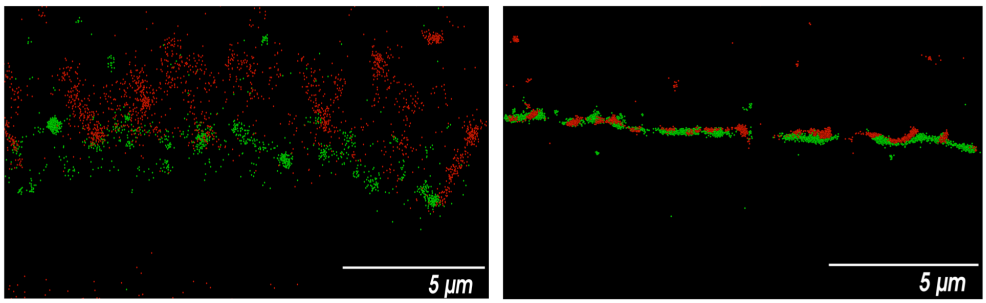

Figure 4: **Super resolution images of SAB neurons in wild type worms where recovery times were compared**. The green are labeled synaptotagmin-1 proteins and the red are labeled RIM proteins and each clustering of color represents a synapse. A) worms recovered for <6 hrs. B) worms recovered for 10 hrs. Background labeling decreases and density of protein localization increases with increasing recovery time.