CP4: Neuronal Connectomics: Edward Boyden, Massachusetts Institute of Technology
Funding Source and Period: NIDA, 5R01DA029639-06, 2010-2019 (Boyden, PI) **Associated With**: TRD1, 2
Significance: Dr. Boyden leads the Synthetic Neurobiology Group, which has developed many cutting-edge techniques for understanding brain connectivity and function. These include optogenetics, automated patch clamp, and expansion microscopy (1-20). One goal of this collaboration is to map the full circuit diagram of the brain (the 'connectome'). Today, the only connectome available is for C. elegans (21). Mapping the connectome is challenging because the typical minimum separation between neuronal membranes, such as at a synapse, is on the order of 20-50 nm. High resolution imaging with an isotropic resolution of at least 50 nm is therefore required. To map the mouse connectome throughout the 0.5 cm^3 volume of the brain, we need to acquire a dataset containing $4x10^{15}$ voxels, which requires super-resolution methods and data analysis algorithms with unprecedented pixel throughput. Most efforts in connectome mapping are based on electron microscopy (22). Although electron microscopy has nanometer-scale resolution, image acquisition is much slower than that of optical microscopy. More importantly, automated image processing of greyscale, complex, electron micrographs is difficult (23), and handling the exabytes of data that would be obtained by electron microscopy of a whole brain is an unmet challenge. We hypothesize that high contrast fluorescent images (where only the neuron branches and synaptic junctions are labeled with high-contrast colors) will overcome the image processing challenges and make the mapping of all the synaptic connections in a mammalian brain possible. This approach demands the development of ultra-high throughput 3D-resolved super-resolution imaging techniques (24-26). If successful, the connectome may provide an underlying ground truth to model information flow in the brain, leading ultimately to an understanding of cognition itself; an important goal of the Brain Initiative / European Brain project. While accurate modeling of whole-brain computation may remain elusive for many years, correlation of functional imaging *in vivo* with local connectivity information, mapped post-mortem even over a small brain region may inform on the structural origin of many neural pathologies.

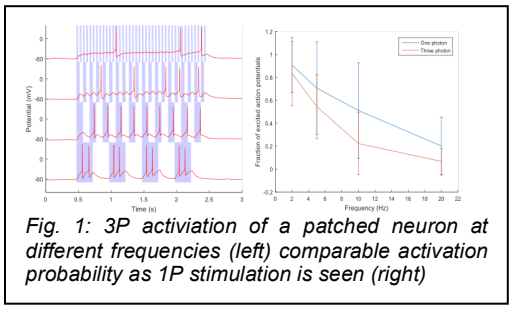

Fig. 1: 3P activiation of a patched neuron at different frequencies (left) comparable activation probability as 1P stimulation is seen (right)

Approach: *Progress Report* LBRC has collaborated with the Boyden lab on several projects over the past two years, making advances in two main areas. First, we have developed a new method (under review in Nature Light: Science & Applications) for targeted stimulation of a single cell in thick tissue by optogenetic activation of opsins (4, 12) using three-photon temporal focusing (27, 28), an extension of previous two-photon approaches (29) (Fig. 1). Second, we have designed and constructed a high-throughput depth-resolved super-resolution RESOLFT system, partly supported by a $200K seed grant from the MIT MINT Program. Instrument construction is underway (Fig. 2) and its optimization is the task of TRD.1.2.

Work Plan This first-generation super-resolution instrument should be available for testing within a year, using cells expressing switchable probes such as Dronpa-M159T (30) or rsEGFP2 (31-33). The Boyden lab will develop expression/labeling and specimen preparation protocols for brain sections in order to optimize the samples for tracing neuronal dendrites, and identify synaptic clefts accurately, *via* strategies such as clearing, expansion (5), and inner membrane leaflet anchoring. The instrument design and specimen preparation protocol will be iteratively improved until we are confident that the system is sufficiently stable to undertake the year-long task of imaging a whole mouse brain.

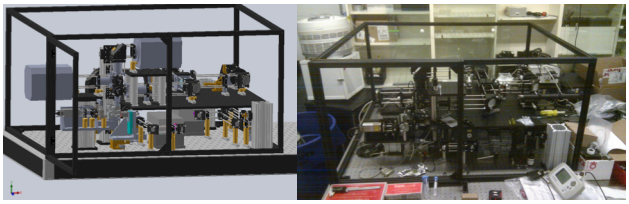

Fig. 2: Connectoscope V1 based on RESOLFT.

Push-Pull Relationship: We **push** volumetric 3D super-resolution microscopy (TRD1.2) to Dr. Boyden's lab to perform connectome mapping. The Boyden lab will **pull** us toward increasing imaging resolution, speed, and widening probe selection. We will further **push** both RESOLFT and STED variants of these systems for evaluation, as the two approaches have different strengths and weaknesses, such as power requirements *vs.* resolution. Beyond *ex vivo* studies, we further envision that the Boyden lab may **pull** us towards developing *in vivo* super-resolution methods for functional studies of living brains or brain slices. Exploring this possibility, the Boyden lab is pioneering methods to improve *in vivo* imaging conditions, by making a live animal brain more transparent. One such approach involves equalizing the refractive index variations in tissue. Towards this goal, we **push** depth-resolved quantitative tomographic phase microscopy (TRD2.2), which will allow the Boyden lab to map the refractive index distribution *in vivo,* assisting in the development of this optical clearing technology. This need will further **pull** us to optimize tomographic phase microscopy for animal work, with the potential for extending this technology to human disease diagnosis.

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