PROJECT SUMMARY (See instructions):

Dendritic spines are tiny protrusions that host more than 90% of excitatory synapses throughout the brain. Although they have been the topic of intense study since their discovery more than a century ago, much remains unknown about their specific functions. Due to their small size (typically <1 μm), imaging spines is technically challenging. Three-dimensional reconstruction from serial section electron microscopy studies have revealed much about the ultrastructure and function of spines, but requires fixed tissue, which makes mechanistic interpretations from 3DEM data challenging because dynamic changes in synapses cannot be observed directly. Two photon fluorescence microscopy allows dynamic imaging of spines, but due to limited spatial resolution, it is rarely possible to quantify subtle changes in spine morphology. More recently, superresolution optical microscopy such as STED has been used to visualize spine dynamics. However, STED requires high laser powers for depletion, which can lead to photodamage and photobleaching. Furthermore, STED is typically limited to superficial structures and cannot image deep into tissue. In this project we will develop a new superresolution optical microscopy technique for imaging spines in intact brain tissues that does not require high laser powers and can image several hundred microns into tissue with tens of nanometer spatial resolution. Our technique will combine two synchronized lasers at different wavelengths with different spatial modes to create improved point spread functions. This two color, mixed mode multiphoton microscopy (2C-4M) technique allows acquisition of two images with different mode combinations whose difference is more than five times higher resolution than standard 2PM. We will use this technique to image morphological changes in dendritic spines induced by LTP. The superresolution optical images will then be compared with 3DEM images of the same structures. This project brings together two established labs in neuroscience and biomedical engineering for the first time in a new collaboration that will lead to significant advances in our ability to visualize synaptic structures.

RELEVANCE (See instructions):

This project addresses a fundamental challenge in mapping neural circuits in the brain. Three-dimensional visualization of neural connections down to the level of individual synapses remains a daunting task. In this project we will develop a new superresolution optical microscopy technique that will enable such visualization deep in brain tissues. Our project brings together labs from Biomedical Engineering and Neuroscience at UT Austin in a new collaboration. These labs have expertise in optical microscopy, in vivo imaging, electron microscopy, neuroscience and cellular mechanisms of synapses. Together this team will develop and apply this novel imaging technique to study synaptic plasticity in the hippocampus. We expect this new microscopy technique to be widely applicable to other fundamental questions in neuroscience in the future.