

PROJECT SUMMARY (See instructions):

Abstract

Improving tools, techniques, and methods for studying the dynamic activity of multiple neuronal populations in brain circuits *in vivo* is one of the major goals of the BRAIN Initiative. The Roberts Laboratory studies a compact song learning-associated neuronal circuit in the zebra finch, called Area HVC, which contains multiple intermingled neuronal populations that differentially process auditory information. A major barrier to understanding how these neuronal populations contribute to song learning is a lack of tools capable of monitoring the activity of each population, each comprising several thousand neurons, in real time. The Meeks Laboratory utilizes a form of light sheet microscopy called objective-coupled planar illumination (OCPI) microscopy that is well-suited to fast volumetric imaging, but which is not currently configured for real time multicolor imaging or *in vivo* imaging. This collaborative proposal will develop a new multicolor OCPI (mOCPI) microscope and deep-tissue OCPI (dtOCPI) microscope, which will overcome some of the significant challenges to *in vivo* HVC research and produce new tools amenable to studying other complex neural circuits.

The two major experimental goals of this proposal involve the design and implementation of mOCPI and dtOCPI. The Meeks Lab will utilize their experience designing and operating OCPI microscopes to support these efforts. mOCPI development will involve the addition of a new light path and sCMOS camera to an existing OCPI design, and will require significant modifications to the custom software that coordinates volumetric image acquisition. The Roberts and Meeks Laboratories will then collaborate to build an OCPI-compatible anesthetized *in vivo* experimental rig and methods for orienting HVC under the OCPI microscope. This will involve integration of a multi-axis head positioner, anesthesia equipment and instruments to regulate animal temperature. Upon completing these tasks, the Meeks and Roberts Laboratories will acquire of multicolor volumetric images of HVC spanning $100,000\ \mu\text{m}^3$ at 1-4 Hz.

The development of dtOCPI will involve the design and implementation of two optical elements, an objective-coupled miniature periscope and an implantable compound GRIN lens/prism. These elements will enable deep high speed volumetric imaging at depths greater than $150\ \mu\text{m}$ from the surface. The Meeks Lab will design and construct the optical elements, and the Roberts Lab will develop surgical methods for stable implantation of the GRIN lens/prism optical element. The two laboratories will collaboratively assess the improvements in imaging depth. Upon completing these tasks, the Meeks and Roberts Laboratories will acquire volumetric images of HVC spanning depths of up to $500\ \mu\text{m}$ from the surface, more than double the current depth resolution of OCPI.

In summary, this collaborative seed grant proposal will generate novel microscopy tools for multicolor, high speed volumetric imaging *in vivo*, enabling currently impossible experiments on sensory processing.

RELEVANCE (See instructions):

The BRAIN initiative seeks to advance knowledge about brain function by supporting next-generation studies of neuronal types and neuronal circuits *in vivo*. The UT BRAIN initiative aims to foster new collaborations in the fields of neuroscience and neurotechnology that make these next-generation studies possible. This proposal involves a new collaboration that matches the light sheet microscopy and engineering expertise of the Meeks Laboratory with the *in vivo* physiology expertise of the Roberts Laboratory. This collaboration will enable real time multicolor volumetric imaging of auditory brain regions in songbirds, and will generate optical tools with broad applicability for studies of other neural circuits. As such, this proposal is well matched to the goals of the UT BRAIN initiative, and will lead to exciting new research by supporting future BRAIN initiative-associated funding opportunities.