PROJECT SUMMARY (See instructions):

Abstract:
Histological and anatomical studies of the mouse inner ear are technically difficult due to its small size and its location embedded in the temporal bone. Even in the most skilled hands, dissection, sectioning and labeling could not be done without moderate amounts of tissue/cell loss. Thus, if we can bypass the existence of the bony structure and visualize inner ear organs and cells, this will contribute greatly to advancing the field of inner ear research. A recently reported protocol, SeeDB, allowed clearing and enabled imaging resolution at the single cell level of the whole brain in mice. Our goal is to modify the SeeDB protocol to achieve 3D visualization of inner ear organs encased in the temporal bone. Our *short-term goal* for this proposal is to modify the existing clearing protocols to achieve good quality visualization of the hair cells and their innervating afferents within the inner ear organs encased in the temporal bone, suitable for subsequent 3-D analysis. Our *long-term goal* is to use this method to study various unanswered questions regarding the morphology of the auditory and vestibular system in many species in health and disease.

Methods:
We will use genetic and mechanical injury models of auditory and vestibular mouse models for applications of the temporal bone clearing technique. First, we will determine the changes in sensory epithelia morphology contributing to auditory and vestibular dysfunction in genetically engineered Usher syndrome mouse model with a lacZ reporter. We will quantify inner ear hair cell damage in a genetic mouse model of hearing loss. Using this non-fluorescent color-labeled model, we will develop the microscopic techniques needed to image individual hair cells and to reconstruct the entire cochlear duct in 3D. Second, we will determine the anatomical changes associated with vestibular ocular response (VOR) abnormalities in caspase-3 mutant mice. We will determine which antibody or fluorescent labeling reagent is most suitable for analyzing the 3D spatial relationship of the vestibular organs within the temporal bone, and to correlate with vestibular function. Third, we will determine vestibular receptor and innervating afferent damage following mild blast injury. We will examine receptor cells, afferent terminals, and afferent fibers by optimizing the most suitable labeling conditions, immunolabels, and clearing conditions to obtain high quality images for 3D reconstruction. We have assembled a strong team with diverse expertise to achieve our goals: genetic mouse models of auditory and vestibular dysfunction (UTMB, Makishima), expert in various animal models in vestibular behavioral studies (BCM, Dickman), confocal and multiphoton microscopy (UTMB, Vargas), and optical core facility at BCM.

Expected results:
We anticipate that the modification of tissue clearing protocols will enable the mouse temporal bone to be suitable for downstream applications. We will for the first time develop methods that allow whole receptor organ morphology to be examined with the entire temporal bone.
The completion of this study will have significant impact on future studies related to models of hearing and balance disorders, because it will build the framework for complete morphological visualization correlated to specific function. Essentially, we have the potential to change the way histology of the inner ear is performed.

RELEVANCE (See instructions):

The combination of advanced optical imaging, molecular biological techniques in clearing tissue, and applying downstream relevant mouse models to study the auditory and vestibular system is novel. The methods can be expanded further to connect the peripheral inner ear studies with central auditory and vestibular pathway studies simultaneously, which was not possible without this technique.

For this funding opportunity, we formed new research partnerships between our lab and 1) Neuroscience Department at Baylor University, Dr. Dickman's lab and 2) Center for Biomedical Engineering at UTMB, Dr. Vargas' lab. Dr. Dickman is a well-established scientist in the field of vestibular research and neuroscience. His lab will contribute expertise on vestibular studies in traumatic brain injury. Dr. Vargas is the Director for Advanced Bio-Optics Imaging Lab, and is an expert in Biomedical Optics and Imaging. She will play a key role in optimizing the imaging techniques and will also assist with clearing techniques. Thus, if successful, this technique can change how anatomical and histological studies in the inner ear will be carried out.