PROJECT SUMMARY:

Background: Acquisition of unprecedented amounts of cellular level data has been made possible by innovative, high-throughput, high-resolution three-dimensional imaging instruments. The *Knife-Edge Scanning Microscope* (KESM), developed and hosted by the PI's research laboratory, is one of the first such instruments currently producing massive three-dimensional data sets. The KESM has been successful in imaging whole mouse brains at submicrometer resolution, revealing the microstructure of the vascular network (stained in India ink) and the neuronal network (stained in Golgi) of the entire mouse brain.

Challenge: The resulting raw image stack data from the mouse brains range over 2 TB per brain, posing serious challenges for geometric reconstruction and analysis, which are needed to turn the raw biological data into information and knowledge about brain anatomy and function.

Key gaps: Automated algorithms exist for tracing the objects of interest (e.g., neurons), but they have limitations in terms of scalability, accuracy, and validation. Most methods (1) are limited to small number of objects in small volumes of data, (2) have fairly high but not high enough accuracy (about 95%), and (3) have limited validation.

Approach and rationale: To address gaps (1) and (2) above, a fast and accurate vector tracing algorithm will be developed based on the project team's on-going work. For gap (3), a selective manual validation framework combined with automated validation based on digital phantoms will be developed, since at the whole-brain scale manual validation is not possible.

Research goals and objectives: The goal of this project is to develop fast and robust vector tracing algorithms tightly coupled with model-based validation. The main objectives of this project are as follows:

1. Develop customized image processing algorithms for serial sectioning imaging.
2. Develop fast, robust tracing algorithms for neuronal and vascular tracing and reconstruction in whole mouse brain KESM data.
3. Develop neuronal and vascular morphology models to obtain statistically accurate models of neuronal and vascular networks.
4. Analyze and model the sectioning and imaging process of the KESM to generate accurate digital phantoms for model-based validation.
5. Develop a large-scale validation framework on (1) manually labeled ground truth from strategically sampled volumes of the data, and on (2) model-based digital phantoms.

Intellectual merit: The microscopic neuronal and vascular data, from the whole mouse brain, along with their accurate geometric reconstruction are expected to open new directions for a truly quantitative research in neuroanatomy (e.g., for use in neural simulators like NEURON and GENESIS). The tightly coupled framework of 3D tracing and model-based automatic validation is expected to serve as a successful model for large-scale data-driven informatics for biological volume data.

Broader impacts: The project will train graduate and undergraduate students, including women and under-represented groups. Excerpts from the resulting data and reconstructions will allow construction of educational databases for the K-12 and the general audience. Data and software will be distributed on an open-source basis. This project includes international collaboration with Dr. Randal Koene at the Fatronik-Tecnalia Foundation in Spain.

Transformative potential: The unprecedented amount and detail in the neuronal and vascular data from this project, along with the accurate, validated quantitative data, can fundamentally change the way brain research is done, and disrupt the common views regarding brain function.

Keywords: (1) whole brain microscopic imaging; (2) neuronal/vascular morphology; (3) tracing and reconstruction; (4) digital phantoms; (5) validation;