PROJECT SUMMARY

Proposed instrument: The goal of this project is to enhance and to transform the Knife-Edge Scanning Microscope (KESM), a "one of a kind" microscopy instrument, into a more widely available system for biological research. KESM is capable of sectioning and imaging whole small animal organs at a sub-micrometer resolution. A prototype KESM instrument was constructed, and its capability was successfully demonstrated by scanning diverse biological organs including whole mouse brains, octopus brains, and the mouse lung at submicrometer resolution. This project aims to enhance this prototype significantly, and transform it into a robust system that can be replicated and operated with ease by other research groups and industry partners.

Relevant need in biological studies: The proposed instrument addresses at least two major emerging directions in biological research: (1) "omics" and (2) multi-scale modeling. With the tremendous success of genomics in the 1990's and beyond, biological research is increasingly moving toward various forms of omics. Many such omics research depends on anatomical information (e.g., connectomics, studying the complete wiring diagram in the brain) or genomic information (e.g., gene expression levels) at the whole-organ-level. Multi-scale modeling has also become a major methodological paradigm in biological research, attested by the formation of the Multi-scale Modeling Consortium, supported by NSF, NIH, DOE, NASA, etc. In many projects in omics and multi-scale modeling, sub-micrometer microscopy data from whole biological organs are essential yet available tools are unable to meet the demand. KESM is expected to fill this gap.

Type of development: Type B (bridging from a "one of a kind" prototype to a broadly available instrument).

Nature of improvement: The improvement on the prototype instrument will focus on "broadening accessibility" with enhanced capabilities:

1. Enhanced imaging: (1) higher resolution optics, (2) fluorescence imaging capability, (3) enhanced linescan camera, and (4) improved illumination through the knife.
2. Enhanced mechanical control: (1) more rigid, higher resolution motion stage, and (2) improved motion control algorithms.
3. Enhanced cutting: (1) reduction of cutting artifact through the use of piezoelectric vibrators and (2) realtime monitoring of cutting-induced vibration (chatter) using accelerometers.
4. Enhanced robustness: (1) realtime imaging error detection and correction, (2) improved pump system for tissue extraction, and (3) enhanced system calibration (focusing and positioning).

The overall resulting improvement can be summarized as follows: (1) 3X improvement in imaging resolution, (2) 10X improvement in robustness of operation, (3) 10X improvement in imaging speed (compared to competing methods), (4) new fluorescence imaging capability.

Broader impacts: (1) *Impact on the research community:* The enhanced KESM will allow researchers to obtain high resolution, whole-organ data for multi-scale, omics investigation of biological organs. (2) *Impact on education:* Microscopic atlases of whole biological organs, such as the web-based KESM mouse brain atlas developed by the project team, can serve as an educational resource for students and educators at all levels (K-12, undergraduate, graduate, and general public). As part of this project, graduate and undergraduate students will be trained in a multidisciplinary environment (neuroscience and computer science). (3) *Instrument dissemination plan:* The project team will collaborate with 3Scan, a a start-up company, to streamline system integration and manufacturing of the KESM instrument for broader dissemination. The design of the instrument and the operational instructions will be made available to the biological research community for those who wish to build their own instrument.