Title: **Digital Muscular and Fascial Anatomy Using Contrast-Enhanced MicroCT**

PI: Tobin Hieronymus, Associate Professor of Anatomy & Neurobiology

Research Location: NEOMED

Abstract: Smooth muscle plays a critical role in the function of many organ systems, but our ability to understand the effects of smooth muscle contraction are limited by our current inability to directly record smooth muscle activity *in-vivo*. Unlike skeletal muscle, smooth muscle does not depend on cell-membrane depolarization to coordinate contraction, so standard techniques such as electromyography (EMG) will not work. Current research in Hieronymus lab is focused on two major aims: (1) applying established methods of measuring brain activity (calcium indicator fluorometry) to the novel setting of peripheral smooth muscle tissues to directly record activity, and (2) developing selective, localized, and reversible interventions to manipulate smooth muscle contraction for functional studies. This summer research project will make use of recent advances in microCT imaging to characterize the architecture of smooth muscle tissue in bird skin (our lab's experimental model system for smooth muscle fluorometry and manipulation), with particular attention to its relationship to the superficial and deep fascia of the human-comparable musculoskeletal elements of the forelimb.

Significance: In addition to the smooth muscle lining the GI tract, airways, and vasculature, avian skin contains bundles of smooth muscle responsible for moving the feathers, similar to the arrector pili muscles in human skin. Because they are abundant and comparatively large, these muscles form a readily accessible experimental model for studies of smooth muscle physiology. My lab is developing a contact fluorometry instrument to record smooth muscle activity *in vivo* and investigating means to locally and reversibly knock down smooth muscle function—both of these aims feed into the broader goal of testing hypotheses of smooth muscle function *in vivo* during normal behavior. Current electrophysiology-based methods do not allow for direct measurement of smooth muscle contraction, and instead rely on indirect measures of correlated skeletal muscle contraction, which are only present in some systems. The ability to directly measure and reversibly manipulate smooth muscle function *in vivo* has implications across urogenital, gastrointestinal, and cardiopulmonary physiology.

Goals & Objectives: The goal of this study is to provide the first tomographic characterization of smooth muscle bundles in avian skin. The student's research experience will focus on assessing interconnections between dermal smooth muscle bundles, superficial fascia, and underlying deep fascia and skeletal muscle of the forelimb. The specific question to be addressed is whether the unusual connections of dermal smooth muscle to underlying bone in the avian forelimb are mediated by deep fascia and associated muscular tissue.

Research Methods: The student will apply Diffusable Iodine Contrast Enhancement (DICE) and Selectively Perfused Iodine Contrast Enhancement (SPICE), both recently developed techniques for imaging normally radiolucent tissues with X-ray computed tomography (CT). These techniques employ the radiodensity of iodine as a contrast agent for CT imaging, and are thus comparable to the use of injectable iodinated contrast media for angiography. Paraffin histology of focal samples will be used to confirm tissue relationships in areas of interest.

Data Analysis: Analysis for this project will include segmentation using Avizo CT analysis software to generate computer models of the musculature and surrounding tissues.

Contribution to Overall Research Effort: Completion of this project will augment continuing experiments with avian feather muscles. This work will also produce a stand-alone set of anatomical results suitable for student-led publication.

Student Fellow Mentoring Plan: Student fellows will take part in weekly lab meetings with all lab members to identify and address lab-wide issues and tasks. Student fellows will also be expected to attend the weekly journal club for the Musculoskeletal Research Focus Area, providing exposure to a broad range of research topics as well as a chance to interact with researchers at different career stages—typical attendance in summer includes 2-4 summer fellows, 2-3 technicians, 1-4 graduate students, 3 postdocs, and 3-4 faculty PIs. Timely completion and reporting of the student fellows' projects will be ensured by weekly one-on-one meetings with the PI, not only to organize work but also to work through main tasks side by side (*e.g.,* worked examples of analysis, drafting, editing and presentation) both during the summer and through the following year as needed. Materials and equipment for the proposed research are currently available in the PIs lab.

Title: **Developing Contact Fluorometry Probes for Smooth Muscle**

PI: Tobin Hieronymus, Associate Professor of Anatomy & Neurobiology

Research Location: NEOMED

Abstract: Smooth muscle tissue plays important functional roles in several organ systems (vasculature, GI tract, pulmonary tract, urinary and reproductive systems), but unlike striated and cardiac muscle, smooth muscle does not generate detectable voltage changes during contraction—electrodes can't be used to investigate smooth muscle function in a manner comparable to electromyography (EMG) and electrocardiography (EKG). In this project, the student will work alongside the PI to build and test prototype contact fluorometry systems with the ultimate design goal of recording smooth muscle activity with genetically-encoded calcium reporters *in vivo* in peripheral tissues. The main focus of the student's research experience will be testing the capabilities of probe designs in an *in vitro* genetically-encoded fluorophore system. Along the way, students will engage in the design process, conduct hands-on fabrication with biocompatible materials, and analyze and interpret readout from the probe system.

Significance: Smooth muscle is responsible for several critical functions across a range of organ systems. Patterns of smooth muscle contraction are likely to be impacted by conditions such as dysautonomia, which is a complication of several disease processes including diabetes and neurodegenerative diseases, but we currently lack the ability to detect and discern altered activation and impairment in smooth muscle *in vivo*. Current state of the art for many organ systems is comparable to cardiology with only heart sounds and echocardiograms but no access to EKG. Developing sensors capable of recording smooth muscle activity is a critical step for researching peripheral signs and mechanisms of conditions like dysautonomia and multiple-system atrophy in model systems.

Goals & Objectives: The overall goal of the research project is the development of a contact fluorometry probe with amplification and analog-to-digital conversion hardware that can be carried by a research animal for *in vivo* recording of labeled smooth muscle activity without restraint or sedation. Fundamental aspects of probe and associated hardware design have already been carried out—the student's research experience will focus on testing and augmenting the signal characteristics of the probe system as it records from genetically-encoded green fluorescent protein (GFP) labeled cells *in vitro*. The specific research questions for the student project will be developed with student input to promote engagement and skill transfer for their future work, but will be centered around ensuring that probe design is feasible for *in vivo* applications.

Research Methods: The student will directly take part in sensor prototyping and fabrication, including familiarization with basic circuit design and building instrumentation with biocompatible materials (etching and soldering flexible PCB, fabricating silicone coatings). This research will also familiarize the student with fundamentals of fluorescent indicator systems (excitation and emission spectra, systems for loading or inducing fluorophores in living systems).

Data Analysis: Analysis for this project will include an introduction to signal processing and hypothesis-testing statistics in R, a widely-used open-source statistical software language.

Contribution to Overall Research Effort: Completion of this project will augment continuing experiments with avian feather muscles. Tests of sensor efficacy will be the basis for a student-led publication on open-source instrument fabrication.

Student Fellow Mentoring Plan: Student fellows will take part in weekly lab meetings with all lab members to identify and address lab-wide issues and tasks. Student fellows will also be expected to attend the weekly journal club for the Musculoskeletal Research Focus Area, providing exposure to a broad range of research topics as well as a chance to interact with researchers at different career stages—typical attendance in summer includes 2-4 summer fellows, 2-3 technicians, 1-4 graduate students, 3 postdocs, and 3-4 faculty PIs. Timely completion and reporting of the student fellows' projects will be ensured by weekly one-on-one meetings with the PI, not only to organize work but also to work through main tasks side by side (*e.g.,* worked examples of analysis, drafting, editing and presentation) both during the summer and through the following year as needed. Materials and equipment for the proposed research are currently available in the PIs lab.