**Streamlining QPLI Acquisition and Analysis by Utilizing a Python-based Framework**

The dermal layer of skin primarily consists of collagen fibers connected by crosslinks which form a matrix responsible for the skin’s mechanical integrity. This microenvironment is under a constant state of tension, resulting in a constant breakdown and synthesis of collagen fibers. The cyclic nature of collagen regulation does not apply to the collagen crosslinks however, which are not as easily broken down, leading to a crosslink-rich microenvironment and a decrease in mechanical strength as a function of age. There have been previous efforts to better understand collagen-mediated structure-function relationships through the use of mechanical testing, and then imaging the collagen structure following the mechanical test, which ultimately results in a lacking understanding of what is occurring to the collagen fibers during the mechanical test.

Collagen fibers have a unique conformation which results in the fibers being naturally birefringent, or containing two refractive indices. Microscopy tools such as quantitative polarized light imaging (QPLI) can probe birefringence, allowing for a pixel-wise measurement of fiber orientation and relative amount of collagen fibers in a relatively fast time frame, and can be done in parallel with other quantitative tests such as mechanical testing. Due to the nature of this process, there are not well established protocols for developing one of these systems, and hardware control is primarily done using MATLAB. By utilizing a commercial, closed source programming language, such as MATLAB, not only do some scientists not have access to the programs written, but some processes within the programs are not well understood. These issues can lead to a program that may or may not run correctly for scientists, as well as have the potential to be inaccurate, which is essential for high-speed image acquisition. **The goal of this study is to utilize open-source, cross platform software to create a QPLI acquisition and analysis workflow.**

Python is an open-source, object oriented programming language that is easy to understand, and is widely accepted in the science community. Furthermore, product manufacturers allow for hardware access in both open source compiled (typically C++) and interpreted (typically Python) languages. Python also allows for groups of programmers to write packages for the software, which allow for even more functionality. An example of one of these packages is OpenCV, which allows for broad camera connectivity and rapid image processing. The *central hypothesis* of this study is that by expanding Python with both proprietary and open-sourced packages, a workflow can be designed for a general QPLI system, which allows for quick and accurate image acquisition and processing. First, the Python-based workflow will be designed with a particular QPLI system in mind (**Aim 1**). Then, the image acquisition and analysis capabilities of the system will be measured to ensure both accurate and precise measurements (**Aim 2**).

**Aim 1: Generate a Python-based QPLI workflow** **by utilizing both proprietary and open-sourced software packages.** QPLI acquisition requires multiple type of hardware to all work in unison. The QPLI system method requires for a linear polarizer to rotate 180 degrees, during which a camera needs to acquire at least three equally-spaced images. As previously described, OpenCV is an ideal candidate for handling the camera acquisition and subsequent analysis. The rotating linear polarizer can be driven by many different types of systems, such as a broken-out Arduino board, or an enclosed rotary stage, both of which commonly have supported packages within Python that are either community or manufacturer based. By completing this aim, production of this type of system will be both highly collaborative and welcoming to scientists.

**Aim 2: Validate the QPLI system workflow to ensure both accuracy and precision for acquisition and analysis processes.** Accuracy and precision are the main driving factors for straying away from closed-source programming languages such as MATLAB. Typically, these type of workflows are hard to understand, and typically produce a large amount of computational overhead that results in slow, inaccurate, and imprecise workflows. To validate the speed and error in the new Python-based workflow, the computational requirements and timing will be measured for both the new workflow, and previously established MATLAB-based workflows. By completing this aim, it will be shown that the new workflow is overall more preferred for this type of system.

**Conclusions:** QPLI is a tool that allows for rapid pixel-wise collagen fiber analysis, which can be utilized in a multitude of studies. However, the generation and implementation of code to accurately and precisely use this type of system is lacking. By completing this study, accessibility of these types of systems will be much improved.