

# IN VIVO SARCOMERE LENGTH MEASUREMENT BY MINIMALLY INVASIVE MICROENDOSCOPY

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## INTRODUCTION

Knowledge of muscle sarcomere lengths is needed to understand of muscle physiology and disease. Unfortunately, individual human sarcomere lengths have never been measured *in vivo* due their small size (1-4  $\mu\text{m}$ ), which is not resolvable with current clinical imaging modalities. We have developed a minimally invasive method capable of imaging individual sarcomeres deep within the muscle of living animals without the use of exogenous dyes. We have used this technique to visualize individual sarcomeres and muscle fibers of mice. Our technique is also applicable to humans, and opens the door to clinical measurement of individual sarcomere lengths.

## METHODS

Muscle produces a nonlinear optical signal during a process called Second-Harmonic Generation (SHG), which is thought to originate from the tail region of myosin heavy chain (Plotnikov et al., 2006). We generated a SHG signal in the muscle of anesthetized C57BL/6 mice (Figure 1A and 1B) using a Gradient Refractive Index (GRIN) microendoscope (Jung et al., 2003). For this study, we used microendoscopes whose diameters ranged from 1000  $\mu\text{m}$  to 350  $\mu\text{m}$  (Figure 1C). These microendoscopes produced acceptable fields of view and allowed us to reach depths of several centimeters within the tissue. We imaged both muscle in its passive state and when activated through stimulation of the tibial

nerve. We measured average sarcomere lengths by fitting a sine wave to the autocorrelation function of an image.

A. SHG imaging schematic B. Microendoscope in mouse leg

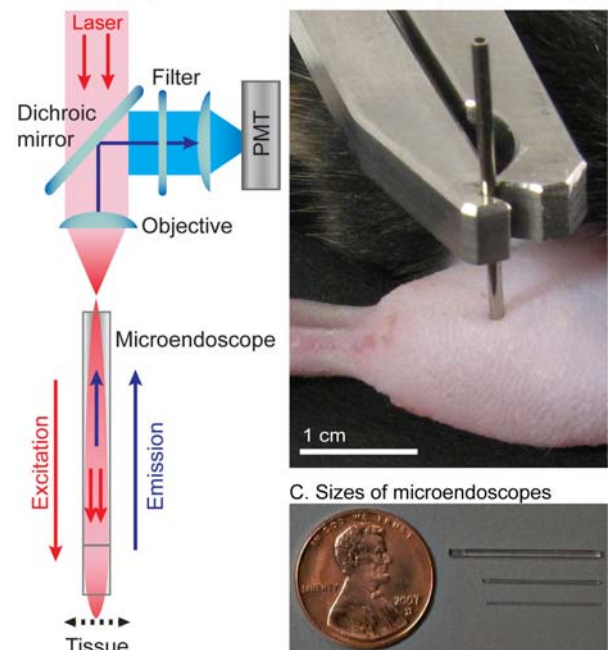
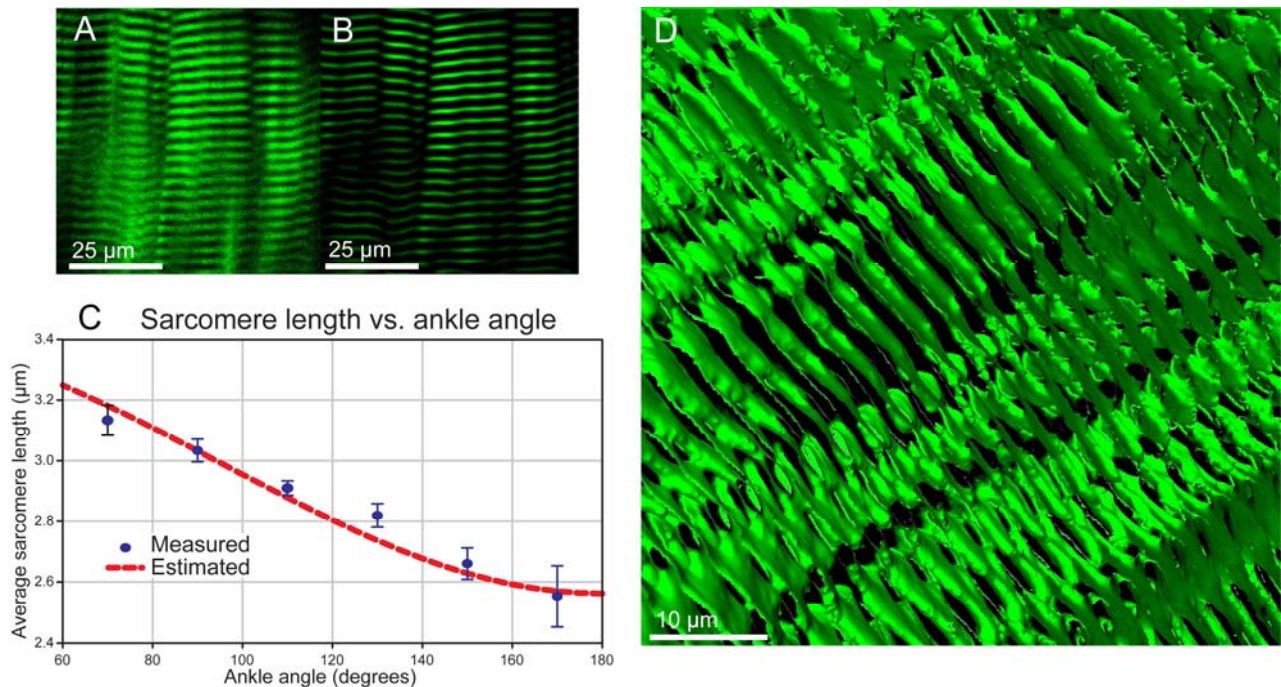


Figure 1: **GRIN microendoscope used to conduct sarcomere imaging.** An infrared pulsed laser is focused by a GRIN microendoscope to excite deep tissue. The same microendoscope collects the emitted SHG signal, which is reflected off a wavelength-specific dichroic mirror through a filter and into a photomultiplier tube (PMT) detector (A). A stainless-steel clad 350  $\mu\text{m}$  microendoscope is inserted in the lateral gastrocnemius of a living mouse (B). The three sizes of microendoscopes (1000  $\mu\text{m}$ , 500  $\mu\text{m}$ , 350  $\mu\text{m}$  diameter.) shown near a coin for scale (C).



**Figure 2:** *In vivo* muscle fibers imaged through a 350  $\mu\text{m}$  microendoscope (A). Image A after it has been band-pass filtered for sarcomere lengths between 1-5  $\mu\text{m}$  (B). Measured and estimated sarcomere length vs. ankle angle for the mouse lateral gastrocnemius ( $n=5$ ); error bars indicate standard error of measurement (C). A benefit of multiphoton imaging is its intrinsic sectioning capability. A 3D rendering of mouse lateral gastrocnemius from a stack of 1  $\mu\text{m}$  thick slices (D).

## RESULTS AND DISCUSSION

We have demonstrated the feasibility of minimally invasive SHG microendoscopy to measure individual sarcomere lengths *in vivo* without the use of exogenous dyes (Figure 2A and 2B). We were able to measure sarcomere lengths from deep within both passive and active muscle tissue. We measured passive sarcomere length vs. ankle angle (Figure 2C) that is consistent with previous studies (Goulding et al., 1997) and estimates based on measured architectural data such as pennation angle, moment arm, and fiber lengths. Additionally, we were able to create high-resolution 3D models of sarcomeres *in vivo* due to the intrinsic sectioning capabilities of multiphoton microscopy (Figure 2D).

For the first time, there is an imaging modality capable of measuring individual sarcomere lengths *in vivo* with minimal invasiveness, opening the door for researchers

to address fundamental questions about muscle physiology. Additionally, because this imaging modality is adaptable to humans, we are looking forward to its use in clinical imaging.

## REFERENCES

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