

## Second Harmonic Generation Imaging as a Virtual Biopsy for Evaluating Nerve Injury in Upper Extremity Trauma: A Cadaver Study

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### INTRODUCTION:

Roughly 5% of patients admitted to level I trauma centers have peripheral nerve injuries (PNIs), and iatrogenic nerve damage is a risk across all orthopedic subspecialties. Stretch injuries represent one of the most devastating forms of PNI, and a timely assessment of injury severity and, if necessary, immediate surgical intervention are key to increase chances for recovery. The appropriate surgical treatment depends on the extent of nerve damage, which is often unknown in incontinuity lesions. Thus, a timely assessment of nerve structure and damage in acute upper extremity injuries is desperately needed. Second Harmonic Generation (SHG) Microscopy effectively visualizes collagenous tissue and showed promising results as an in-vivo assessment of nerve injury in rodents but has yet to be tested on human nervous tissue. This human cadaver study investigates two questions: (1) Can non-invasive SHG-Imaging identify and distinguish the individual collagenous substructures in human radial, median and ulnar nerves? (2) Can SHG-Imaging detect structural damage in human nerves following destructive in-situ stretch injury?

### METHODS:

Eight (n=8) fresh frozen upper extremities were thawed at room temperature for 12 hours and equally divided into two groups: A no-injury (NI) and a load-to-failure (LTF) group (n=4 arms each). In LTF specimens, the median and ulnar nerves were surgically exposed and gently separated from surrounding tissue in the forearm, while the radial nerve was exposed in the dorsal upper arm. Proximal nerve ends in the axilla were secured in place using sandwich clamps attached to an immobile metal bar. Arms were placed on an Instron material testing system. 8cm of each exposed nerve was secured under two blunt metal pins. Mimicking a displaced fracture edge, a metal hook was placed beneath the nerve and raised at a speed of 0.5mm/s until complete nerve transection occurred. After the experiment, nerves were excised along their full length. NI- and LTF-nerves were placed in isotonic saline below a custom-made 3 by 4 cm glass cover slip and positioned under an FVMPE-RS Multiphoton Microscope using a laser-wavelength of 900 nm to induce an SHG-signal. 240 µm deep z-stack images were acquired using a bandpass-filter at a wavelength of 450 nm. Imaris software generated 3D reconstructions. Nerves were fixed in 10% Zinc-Formalin and embedded in acrylic resin for high-fidelity histology.

### RESULTS:

H&E-stained histology sections of NI-nerves exhibited normal nerve architecture with loose superficial collagen fibers and densely packed, highly organized collagen layers deep within the epineurium, which was pervaded by longitudinal threads of adipose tissue. Nerve fascicles were surrounded by a thin perineurial layer and contained nerve fibers embedded in endoneurial tubes. All collagenous substructures visible on histology were clearly identifiable and distinguishable on SHG images of uninjured nerves. On visual inspection, LTF-Nerves exhibited macroscopic disruption of the epineurium and exposed fascicles proximal and distal to the zone of full transection. Epineurium rupture and exposed fascicles were clearly identifiable on SHG-Imaging. No sheath surrounded exposed nerve fibers, indicating joint rupture of the epi- and perineurium. Epineurial collagen of LTF-nerves appeared heavily disorganized, with short fiber fragments following no clear trajectory. The epineurial SHG-signal of injured nerves was less intense than that of undamaged nerves. Findings were consistent amongst all three nerve types assessed in this study.

### DISCUSSION AND CONCLUSION:

To the best of our knowledge, this is the first study to visualize human nervous tissue using SHG Microscopy. The collagenous framework of peripheral nerves is paramount in guiding nerve fiber growth and limiting axon overgrowth, preventing formation of traumatic neuromas.<sup>5</sup> Damage to this framework drastically increases the risk for regeneration failure, neuroma formation and chronic dysfunction. Our findings demonstrate that non-invasive SHG-Imaging offers a detailed visualization of all collagenous substructures of the radial, median and ulnar nerve and detects structural damage, such as epineurial collagen disorganization, disruption of the epineurium and exposure of individual nerve fascicles in unprecedented detail. SHG Microscopy provides a quick and non-invasive alternative to a histological biopsy, which inherently damages the nerve.

A detailed intraoperative assessment of a nerve's collagenous structures could guide the treatment for upper extremity nerve injuries and detect nerve damage that requires immediate surgical intervention, allowing for a more informed surgical decision making process.

