

# Evaluating Human Articular Cartilage Function via Second Harmonic Generation (SHG) Imaging

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**INTRODUCTION:** Many arthroscopic tools developed for knee joint assessment are contact-based, which is challenging for in vivo application in narrow joint spaces. Second harmonic generation (SHG) laser imaging is a non-invasive and non-contact method for assessing cartilage integrity, thus presenting an attractive alternative. However, the association between SHG-based measures and cartilage mechanical properties is not well established. Here, we investigated the feasibility of using image-based measures derived from SHG microscopy for objective evaluation of the cartilage quality and mechanical properties.

**METHODS:** This study received ethical approval from the University of Calgary Conjoint Health Research Ethics Board and Alberta Health Services. Fresh tibial plateaus were collected from total knee replacement surgery patients (N = 9; 2 males, 7 females; mean age: 64.1 ± 7.5 years) and preserved in a phosphate-buffered saline (PBS) solution. Swiftly transported to the university lab, the samples underwent testing as outlined in Figure 1. Mechanical properties were assessed through indentation stiffness ( $E_{inst}$ ) testing and a streaming potential-based quantitative parameters (QP). The relationship between cartilage electromechanical properties ( $E_{inst}$  and QP) and image-based metrics from SHG imaging, tissue thickness, and cell viability was studied using correlation and logistic regression analyses. SHG-related parameters encompassed the volumetric ratio of organized collagen networks ( $\Phi_{col}$ ) and the coefficient of variation in SHG intensity ( $CV_{SHG}$ ). SPSS software (version 27, SPSS Inc. IL, USA) was used for all statistical analyses. Earlier research established significant correlations between electromechanical QP and the International Cartilage Repair Society (ICRS) grade<sup>1</sup>. Consequently, the present data were categorized into three groups based on QP values (group 0: QP ≤ 13, group 1: 13 < QP ≤ 21, and group 2: QP > 21), aligning with ICRS grades 0, 1, and 2, respectively.

**RESULTS:** Figure 1 graphically displays the correlation between tissue stiffness ( $E_{inst}$ ), electromechanical traits (QP), cell morphology (green ellipse), and the volume fraction of organized collagen networks (grey signal). Notably, regions with low  $E_{inst}$  and high QP (blue circle, Fig. 1) exhibited scant live cells and diminished  $\Phi_{col}$ . Conversely, areas with high  $E_{inst}$  and low QP (red circle, Fig. 1) displayed chondrocytes with typical elliptical shapes and elevated  $\Phi_{col}$ . Cartilage degeneration led to significant changes in  $E_{inst}$ ,  $\Phi_{col}$ , and  $CV_{SHG}$ , whereas cell viability and tissue thickness remained relatively consistent across all tissue groups (Fig. 2).

**DISCUSSION:** This study was aimed at assessing SHG imaging's potential for objectively quantifying human articular cartilage quality. Our data validates the established inverse relationship between instant stiffness ( $E_{inst}$ ) and electromechanical QP (Fig. 2) from earlier work<sup>1</sup>. Both  $E_{inst}$  and QP reflect cartilage function, demanding tissue indentation for evaluation. In contrast, SHG imaging provides non-contact measurements, potentially impactful for future clinical applications. While multiphoton SHG imaging unveils tissue structure, its relevance in precisely characterizing cartilage mechanical properties remained untested. We bridged this gap by correlating SHG-derived metrics ( $CV_{SHG}$  and  $\Phi_{col}$ ) with human cartilage electromechanical properties. Our results show that (i) the  $\Phi_{col}$  correlated strongly with  $E_{inst}$  ( $\rho = 0.97$ ,  $p < 0.01$ ), and QP ( $\rho = -0.89$ ,  $p < 0.01$ ), (ii) the  $CV_{SHG}$  also correlated with  $E_{inst}$ , and QP though with less strength ( $|\rho| = 0.52 - 0.58$ ), (iii) by using QP scores to group the tissues into regions of degrees of degeneration,  $E_{inst}$  and  $\Phi_{col}$  were found to be the most sensitive predictors of cartilage health quality, whereas  $CV_{SHG}$  only showed moderate sensitivity, and (iv) cell viability and tissue thickness, often used in the past, were poor predictors of cartilage quality.

**SIGNIFICANCE/CLINICAL RELEVANCE:** We introduce a straightforward and reliable image-based method for evaluating cartilage's load-bearing mechanical properties and overall health. The parameter  $\Phi_{col}$  from imaging exhibited strong correlations with tissue stiffness and streaming potential indicators. This finding suggests a promising avenue for the use of SHG imaging for clinical application in assessing joint health, and they have the potential to inspire the orthopedic community to delve deeper into utilizing multiphoton laser imaging for early detection of osteoarthritis in the human knee.

**REFERENCES:** 1) Sim et al., Ann Biomed Eng 45, 2410–2421. <https://doi.org/10.1007/s10439-017-1879-4>

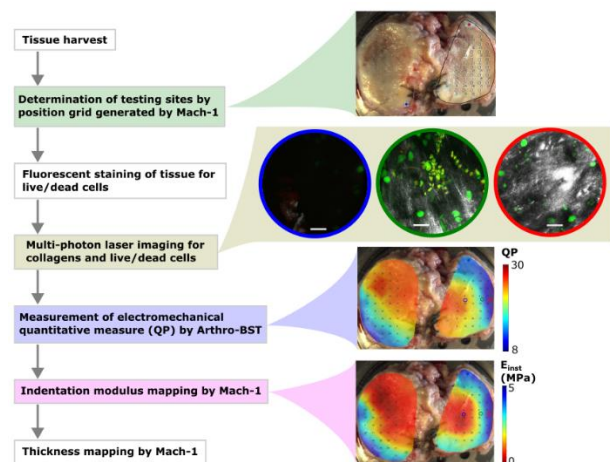


Fig. 1. Workflow of the current study. Representative example showing the relationship between tissue stiffness ( $E_{inst}$ ), electromechanical properties (QP), cell shape (green ellipse), and volume fraction of organised collagen network (grey signal) are shown at different locations of the joint surface marked by blue, green and red circles. Scale bar: 40 μm

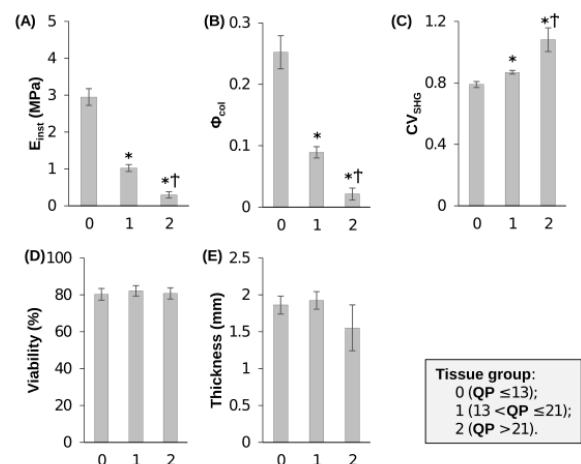


Fig. 2. Estimated marginal mean ( $\pm 1$  SE) values of (A)  $E_{inst}$ , (B)  $\Phi_{col}$ , (C)  $CV_{SHG}$ , (D) cell viability, and (E) local tissue thickness, as a function of tissue group (0–2 in the order of increasing disease severity, with group 0 considered a healthy tissue region). The values  $E_{inst}$ ,  $\Phi_{col}$ , and  $CV_{SHG}$  changed significantly with cartilage degeneration, whereas cell viability and tissue thickness remained unchanged with tissue groups. \* and † indicate statistical difference when compared with tissue group 0 and group 1, respectively.