Quantitative Polarized Light Microscopy of Human Articular Cartilage: Technique Description and Error Analysis

Schoenhoff EK, Chen AC, Raub CB, RL Sah.
Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093-0412
Tel: 858-534-5682 E-mail: eschoenh@ucsd.edu

Summary: Polarized light microscopy was performed on human articular cartilage. Image processing resulted in quantitative metrics of collagen network microstructure. Error analysis performed, and microstructural parameters are presented for normal and degenerate human cartilage.

Introduction: Osteoarthritis (OA) is a very prevalent joint disease, yet the underlying structural changes to cartilage during degeneration and OA are only partially known. Structural changes in the collagen network have been linked to age-associated degeneration [1]. A greater understanding of the role of the collagen network in cartilage degeneration and OA is important to developing targeted therapies. Quantitative polarized light microscopy (qPLM) is a technique which quantifies collagen network microstructure. The purpose of this study was to perform qPLM on human cartilage and assess error due to instrumentation and technique.

Methods: qPLM microscopy was used in order to determine the mean orientation angle (α) and parallelism index (PI) of collagen network in human articular cartilage, and error was assessed from background, sample orientation, and instrumentation. Fixed sections of human articular cartilage mounted on slides were deparaffinized and imaged with a Nikon Optiphot-2 and LOMO Polam-213. Images were recorded with the two different cameras at equal pixel resolution of ~6.6 µm. Images were collected and processed, and the PI and α were calculated (using Matlab) at each pixel as previously described [2]. Three background subtractions were performed on the Optiphot images to determine the optimal method: a second-order polynomial background subtraction (PolyFit) and an image-based background subtraction based on scaled (Scaled) and full (Full) background images. To determine the sensitivity of PI and α to background, constants of 20-100 were added to each of the birefringence images, and PI and α colormaps were computed and compared. To test the robustness of α values to sample placement in the image plane, the sample was imaged at imposed angles of 2, 5, 10, 20, and 35°, α correction applied to set values with respect to the articular surface, and results compared to the corrected α colormap at 0°.

Results: Colormaps of PI and α are presented in Figure 1, and RMS with respect to LOMO colormaps. Polynomial background subtraction provided the lowest RMS for Optiphot colormaps. RMS increased linearly with added background level for PI colormaps (25%/100 a.u.; R²=0.97), but not for α colormaps (1%/100 a.u.; R²=0.27).

![Figure 1. Table of α and PI colormaps for a human osteochondral section (medial femoral condyle) generated with the LOMO system (A,B) and the Optiphot system (C-H) and background corrected with a scaled Polynomial Fit (PolyFit; C,D), scaled background images (Scaled; E,F), and full intensity background images (Full; G,H).](image)

The orientation correction method produced RMS ranging from 10% to 33%, and average difference angles from 3.3° to 13.5°, for imposed angles of 2 to 35°. α from the superficial (SZ) and middle zones (MZ) correlated positively with histopathological score, and negatively with structural stiffness (slope test, p<0.05).

Discussion: PI was sensitive to background subtraction and added background; the PolyFit background was best for Optiphot PI colormaps, consistent with higher and nonuniform background with the Optiphot than the LOMO system. α was insensitive to background subtraction and robust to sample tilt up to ~20°. Larger SZ and MZ PI and α values corresponded to markers of cartilage degeneration (increased histopathological score and reduced stiffness). Therefore α and PI may provide useful information about collagen network age-related degeneration.