Second-harmonic generation (SHG) has been established as a useful contrast mechanism in optical microscopy. Based on the non-linear response of a material to an external electric field, the inherent non-centrosymmetric structure of tissues can be induced to generate a second order, non-linear optical signal at exactly twice the frequency of the incident field [1]. Individual noncentrosymmetric molecules will generate a second-harmonic signal, but molecules arranged in a crystalline array will give a very much stronger response. Collagen is an important structural and functional protein in cartilage and the collagen molecule is nonsymmetric and arranged in a triple helix. Therefore, SHG can be an exquisitely sensitive tool for detecting and imaging collagen at high resolution [2].

Here we demonstrated the collagen morphology imaged by SHG microscopy in the superficial zone of kangaroo cartilage from femoral head and distal humerus. The superficial zone showed an interwoven collagen bundles when imaged in a top-down view. The network interwoven collagen bundles in the most superficial zone aligned spatially in a plane parallel to the articular surface. And the cartilage from femoral head displayed bigger bundles of collagen than trochlea of humerus. Furthermore, texture analysis indicated trochlea of humerus is higher in image regularity and homogeneity than femoral head.