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## **Second Harmonic Generation (SHG) microscopy of articular cartilage to image collagen modifications caused by osteoarthritis.**

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Osteoarthritis is a debilitating and painful disease involving the dysfunction and degradation of joint tissue. It affects mainly the knees and the hips and the most widely recognised change in the cartilage is the loss of the proteoglycans and the degradation of the articular cartilage. The irreversible stage of this degeneration is thought to be damage to the collagen type II meshwork, which leads to a cascade of degradation. No cure has been found yet to treat osteoarthritis and when the degradation is too advanced, the only solution is the replacement of the joint. The collagen type II is a protein that has a noncentrosymmetric structure that allows the generation of second harmonic signal. Therefore, second harmonic generation (SHG) microscopy is an ideal technique for high spatial resolution imaging of cartilage. Here, we use SHG microscopy to image the modification of the collagen structure on the surface of articular cartilage and in the deep cartilage matrix. Thick slices of cartilage taken from bovine knee have been used to image the surface of cartilage. In samples taken from diseased knee near localised eroded regions, many structures associated with osteoarthritis has been found. Among them, we found progression of degradation front, entanglement of the collagen matrix and formation of collagen bundles. Using thin sections of human tissue with early stage disease, we have measured the SHG intensity and the polarisation dependence of the signal in deep cartilage matrix in the forward and in the backward direction. Forward refers to the direction of laser propagation while backward is at 180 degrees. By imaging these tissues, we found regions of high forward SHG signal in which the polarization angle at maximum amplitude sometimes differs from elsewhere in the tissue. While the signal in the diseased regions in the forward direction significantly increases, the signal in the backward direction only slightly decreases. Also, the modification of the angle of the polarisation in which the signal is maximum in the backward direction remain the same as elsewhere in the collagen meshwork, unlike what has been observed in the forward direction. Our studies reveal that SHG microscopy is a powerful technique to image the collagen structure in articular cartilage and allows probing the early stages of osteoarthritis.

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