The effect of fibrillar ordering on polarization resolved second harmonic generation

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Second harmonic generation (SHG) microscopy has been widely used to image collagen in a wide array of tissues and for a wide array of pathological applications. There are different types of collagen molecules that all have the triple helical structure in common but vary in their polypeptide composition. This variation in composition leads to variation in structural assembly. E.g. collagen types I and II form aggregates that form semi-crystalline fibrils whereas type IV assembles in a mesh-like structure. As SHG requires a non-centrosymmetric organization of the molecules only collagen types which form fibrils can be imaged.

For many applications it would be advantageous to be able to easily differentiate between collagen I and II. This is especially true in tissue engineering and surgical repair of cartilage. Native cartilage consists of collagen II. Repair cartilage on the other hand, is usually a mixture of collagen I and II (fibrocartilage) and has inferior mechanical properties compared to native cartilage.

As the second order susceptibility between collagen I and II might be different, SHG might be used as a marker of collagen I versus collagen II. Several publicatioins have indicated that this is possible[1, 2]. However, as SHG is a coherent process, the signal is very sensitive to the ordering and orientation of fibrils in the collagen volume. Furthermore, most models used to fit experimental data, make assumptions about cylindrical symmetry and in-plane fibril orientation, assumptions that are often not met in real tissues.

We use numerical simulation, corroborated with experimental data, to indicate that the orientation of fibrils and and the degree of ordering has large impacts on the estimated parameters when using models that are typically employed. Pixels within the image that likely meet the model assumptions of cylindrical symmetry can be found by selecting those that meet the requirement that $\frac{\chi_{31}}{\chi_{51}} = 1$. For these pixels, the experimental measurements indicate variations in susceptibility ratios on the order of 0.02. The results indicate that for most pixels, the effect of the variation in orientation and ordering will create much larger variations in estimated susceptibility ratios and will mask any difference that are due to molecular differences between collagen I and collagen II.

References

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