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Near-field Effects on SHG Imaging

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Collagen fibrils can be found in a wide array of biological tissues such as bones, tendons, arteries, cornea. They give most of the biological tissues its mechanical properties and as such are an important area of research. Second Harmonic Generation (SHG) is a non-linear optical process that is particularly strong in collagen fibrils due to their unique chiral molecular structure, enabling label-free imaging. Furthermore, the radiation pattern correlates with the fibril sizes and orientations in collagen tissue[1], allowing one to study the underlying structure through careful modelling[2], even though the fibril diameters are usually smaller than the SHG wavelength. One key property that is often used for this is the ratio between the far-field signals in the forward and the backward directions, the F/B ratio[3], as that is linked to fibril size.

Our calculations show that the presence of small differences in the refractive index, as commonly found in biological tissue, can have a profound effect on the measured signal of a single fibril. Near-field enhancements cause the SHG signal to come from an area with a smaller radius than the fibril's geometric radius, significantly altering phase matching conditions for the backward scattered signals. This may skew diameter measurements such as in [3]. It also affects imaging of collagen tissue where a entire distribution of fibrils is in the laser focus. The signal of the smaller fibrils will not change significantly, but of large fibrils it will and those can be present in large numbers in collagen tissue. Therefore SHG experiments concerning the direct measurement of fibril diameters need to take these effects into consideration.

References

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