Improved second harmonic generation anisostropy for collagen organization analysis in tissue samples

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Imaging tissue samples by polarization-resolved second harmonic generation microscopy provides both qualitative and quantitative insights into endogenous structures without the need of staining. Due to its coherent nature, the second harmonic intensity dependence on the laser polarization is sensitive to the structure and arrangement of the molecules that produces the signal and offers additional information and means of contrast beyond intensity-based second harmonic generation microscopy. By applying polarization-sensitive measurements different metrics such as the anisotropy factor (β) can be computed and used for quantitative image analysis. The anisotropy factor β is a pixel-wise measure of the alignment of molecular dipoles relative to the incident laser polarization and provides additional information on collagen arrangement not available with intensity measurements alone.

Current established approaches are limited to calculating the anisotropy factor for only a particular laser beam polarization. The anisotropy factor, previously used for cancer assessment in a wide range of experiments, follows a laser beam polarization dependence which is similar to that of the second harmonic intensity and no general guidelines on how to select the best laser beam polarization have yet been defined. We have introduced a novel methodology for selecting the optimal laser beam polarization for characterizing tissue samples using the anisotropy in the purpose of identifying cancer signatures. We combine the second harmonic generation anisotropy analysis [1] with the collagen orientation index computed by Fast Fourier Transform analysis of the recorded images to establish a framework for choosing the laser beam polarization that is optimal for an accurate interpretation of polarization-resolved second harmonic generation microscopy images and anisotropy maps, and hence a better differentiation between healthy and dysplastic areas.

We have demonstrated the proposed methodology for skin tissue stained with hematoxylin and eosin. Our study enhances the application of polarization-resolved second harmonic generation microscopy towards quantitative label-free imaging of collagen in tissue samples.

[1] R. Hristu, S.G. Stanciu, D.E. Tranca, G.A. Stanciu, *J. Biophotonics* **1–9** (2016) / DOI 10.1002/jbio.201600197

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