Background: Second harmonic generation (SHG) / two-photon excited fluorescence (TPEF) microscopy is commonly used for the quantitative assessment of liver fibrosis; however, the accuracy is susceptible to sampling error and count error due to disturbances induced by some forms of collagen in liver specimens.

Methods: In this study, we sought to improve the accuracy of quantitative assessments by removing the effects of this disturbing collagen and optimizing the sampling protocol. Large liver resection samples were scanned using SHG/TPEF microscopy with multiple adjacent images. During the quantitative assessment, we then removed SHG signals associated with three types of extraneous physiological collagen: large patches of collagen near the boundary of the capsule, collagen around tubular structures, and collagen associated with distorted vessel walls. The optimal sampling protocol was identified by comparing scans from regions of interest (ROI) of various sizes (3*3 tiles and 5*5 tiles) with full scans of the same tissue.

Results: Our results demonstrate that the quantitative assessments of liver fibrosis can be greatly enhanced in terms of accuracy and efficiency through optimal sampling and the automated removal of disturbing collagen signals.

Conclusion: These types of algorithm could be integrated in next-generation SHG/TPEF microscopic systems.

The following people have nothing to disclose: Kai-Wen Huang, Kai-Wen Huang

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