Standardized quantification on liver fibrosis using second harmonic generation and two-photon microscopy

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1. BACKGROUND
Determining the extent of liver fibrosis has clinically been difficult due to the lack of a simple, objective method that can accurately quantify the amount of collagen in the diseased tissue. Second harmonic generation (SHG) microscopy has been shown to produce bright and robust signals from non-centrosymmetric fibrillar collagen. We designed a SHG system that can objectively quantify liver fibrosis in an animal model in an efficient, standardized and reproducible manner.

2. METHOD
SHG and two-photon microscopies were performed on livers harvested from bile duct ligated Wistar rats using a confocal microscope with a mode-locked Ti:Sapphire laser. Images acquired were later analyzed with a custom-developed algorithm, producing a collagen index (Fibro-C-index).

3. RESULTS
A linear correlation between Fibro-C-index and the amount of tissue type I collagen was obtained. Fibro-C-index results agree with pathologist scoring closely, which validates our quantification approach. Furthermore, using the Fibro-C-index, early fibrosis (score 1), undifferentiable by light microscopy, showed up to 400% difference in levels of collagen.

4. CONCLUSION
Fibro-C-index provides sensitive measurement for liver fibrosis and accurately reflects the progression of liver fibrosis, especially in early stages. Fibrosis development is easily quantified using a stain-free technique and a fully-automated algorithm, and is shown to be a standardized index system.