

# SHG/TPEF Microscopy Imaging in the Fibrosis and Steatosis Progression of Nonalcoholic Fatty Liver Disease (NAFLD) in Mouse Models



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Animal models of NAFLD provide crucial information not only on elucidating pathogenesis but also in examining therapeutic effects. However, an appropriate scoring system (such as the Brunt system) has not been developed for animals.

To evaluate fibrosis and steatosis progression in NAFLD mouse models with an automated and fully quantitative technique using stain-free Second Harmonic Generation (SHG) / Two-photon Excitation Fluorescence (TPEF) microscopy.

6-week-old male C57BL/6 mice were randomly divided into six groups: (1) High fat diet (HFD) group, 45% HFD; (2) HFDF group, HFD supplemented with fructose in drinking water; (3) HFDF+CCl4 group, HFDF plus intraperitoneal injection of CCl4, twice a week; (4) Western diet (WD) group; (5) WDF group, WD with 15% weight/volume fructose in drinking water; and (6) WDF+CCl4 group, WDF plus intraperitoneal injection of CCl4, twice a week. Liver tissue specimens from the abovementioned NAFLD models and normal diet control mice were collected at several time points.

All tissues were serially sectioned at 4 μm thickness for histological scoring with hematoxylin-eosin and picosirius red staining, as well as SHG/TPEF imaging. The fibrosis and steatosis progression were analyzed by SHG/TPEF quantitative parameters with respect to timepoint and histological scoring using the Brunt system.

Based on their high correlation with the histological scoring and time of modeling, four shared parameters (#LongStrPS, #ThinStrPS, #ThinStrPSAgg and #LongStrPSDis) were selected to create a linear model that can accurately identify differences among fibrosis stages (AUC: 0.8-1,  $P < 0.05$ ) and timepoints (0.67-1,  $P < 0.05$ ) (Figure). These AUC values were mostly higher than using total collagen proportionate area in differentiating fibrosis stages (0.8-1 vs 0.49-1) and timepoints (0.67-1 vs 0.42-1). Also, good correlations were observed between the histopathology and SHG/TPEF assessments of steatosis in all mouse models with Spearman correlations of 0.769-0.931,  $P < 0.05$ .

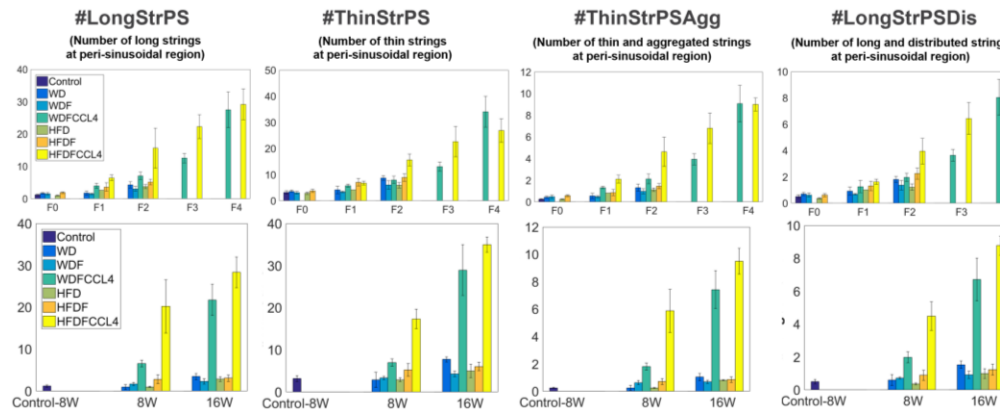
Quantitative assessment using stain-free SHG/TPEF technology could differentiate both fibrosis and steatosis progression in mouse models of NAFLD. An automated quantitative assessment, which combined four shared parameters, correlated well with fibrosis stage and time of modeling, it could be useful to sensitively and specifically monitor liver fibrosis changes in NAFLD mouse models.

This work was supported by the China National Science and Technology Major Project for Infectious Diseases Control during the 13th Five-Year Plan Period (2018ZX09201002-001), and National Natural Science Foundation of China (NSFC) (81870407).

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The authors have no conflict of interest.

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**Figure.** Changes of four shared fibrosis parameters among the six NAFLD mouse models.