

## Automated, fully quantitative analysis by using second harmonic generation microscopy in the fibrosis progression of nonalcoholic fatty liver disease

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*Fatty liver disease: Clinical aspects*

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### Automated, fully quantitative analysis by using second harmonic generation microscopy in the fibrosis progression of nonalcoholic fatty liver disease

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**Background and Aims:** Liver Fibrosis was reported to be associated with Long-term outcomes of patients with Nonalcoholic fatty liver disease (NAFLD). The conventional histological assessment of fibrosis is semi-quantitative which may not sufficiently reflect minor changes of fibrosis. The aims of this study was to evaluate collagen parameters by second harmonic generation/ two photon excitation fluorescence (SHG/TPEF) in adult NAFLD patients and MCD mice models, and develop a automated quantitative evaluating system for fibrosis progression of NAFLD.

**Methods:** 60 MCD mice samples and 62 adult NAFLD biopsies and were collected, stained and scored using Brunt scoring system. SHG/TPEF were imaged and quantified 128 collagen parameters including portal, central and perisinusoidal collagen in liver tissue. The automated quantitative evaluating system was established by employing Brunt scoring fibrosis staging as standard references, and was compared with the collagen proportionate area (CPA) and hydroxyproline (HYP).

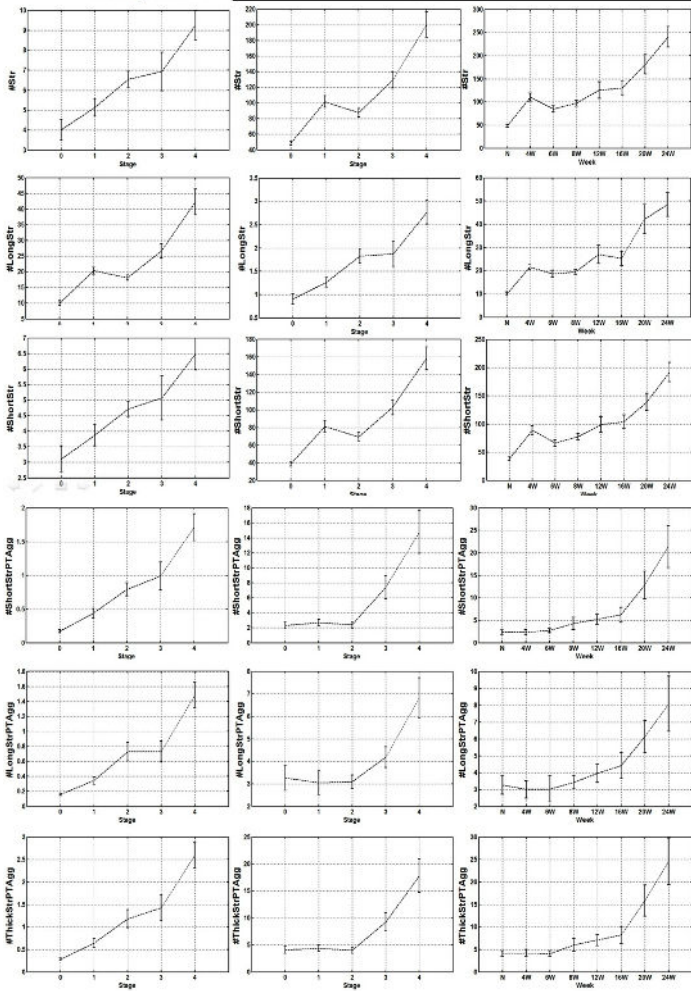
**Results:** First, 15 parameters in NAFLD biopsies and 24 parameters in MCD mice samples, mainly from string collagen, were selected based on their highly correlated with the fibrosis staging. 34 parameters in MCD mice samples were selected based on their highly correlated with different timepoints. To identify the most important parameters from the parameters extracted, we designed a class-specific ensemble feature selection framework, 6 shared parameters (#NoStr, #ShortStr, #LongStr, #ShortStrPTA, #LongStrPTA, and #ThickStrPTA) were demonstrated to identify differences among fibrosis stages with high AUC accuracy (Adult: 0.853-0.982; mice:0.861-0.986), and among different timepoints with high AUC accuracy (AUC values: 0.833-0.993) (Fig. 1). Also the AUC values of 6 shared parameters in MCD mice model were maintained higher than CPA (0.665-0.943) and HYP (0.748-0.968) among fibrosis stages, and different timepontis (CPA: 0.583-0.945; HYP: 0.761-0.972).

**Conclusions:** A automated quantitative evaluating system, combined 6 shared collagen parameters, could be useful to specifically and accurately monitor liver fibrosis in a quantitative manner in adult patients and MCD model with NAFLD. This more detailed, quantifiable collagen parameters based on SHG/TPEF might be applied to assess the efficacy of anti-fibrotic drugs in clinical research in future.

**Figure:**

Adult patients

MCD models



Disclosure of Interest: None Declared