**WED-236**

# Artificial Intelligence analysis of liver biopsies in pre-cirrhotic NASH: qFibrosis explained

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**Background and Aims:** Liver histopathology is a primary outcome for non-alcoholic steatohepatitis (NASH) candidate drugs registration. Besides biopsy size, tissue homogeneity, and staining quality, intra and inter observer variability confound the interpretation of available semi-quantitative scales for fibrosis. We designed an innovative processing and analysis of digitized liver tissue and submit it to artificial intelligence analysis to provide a more sensitive and fully quantitative analysis of fibrosis.

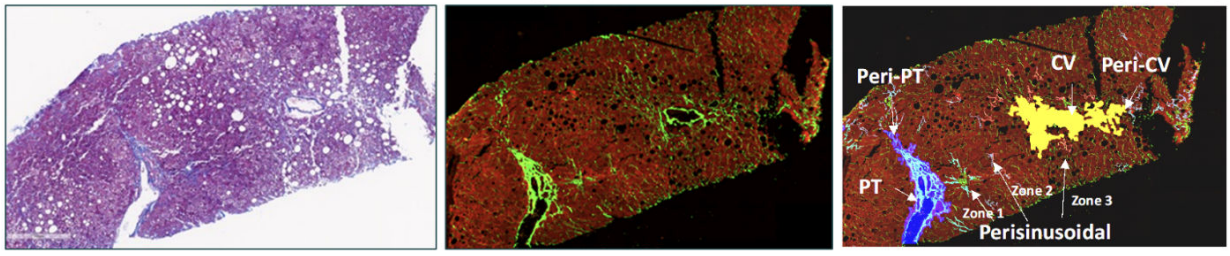
**Method:** Unstained tissue obtained from liver biopsy are digitized using a proprietary system and submitted to two detection methods (Genesis®200): a second harmonic generation (SHG) microscopy to identify collagen and a laser two-photon excited fluorescence (TEPF) microscopy to identify hepatocytes. A fully automated machine-learning (ML) image analysis processes information. Algorithms identify portal, septal and fibrillar collagen and exclude portal tracts and central veins pixels, as well as sinusoidal spaces, and extract multiple features of tissue fibrosis. Finally, for each biopsy tissue, a fully quantitative value, qFibrosis, is obtained. Quantitative and semi-quantitative results are compared.

**Results:** A total of 17 collagen features were selected by ML for the qFibrosis equation, among which, the most important features (with largest coefficients) are number of intersections and number of short and aggregated fiber in the overall tissue region. qFibrosis has open-ended ranges, but typical values are between 0 – 3.7 in pre-cirrhotic NASH (F0-F3) patients, with higher values representing increasing severity of fibrosis. The correlation of qFibrosis values correlate with semi-quantitative fibrosis scores and are 0.761, 0.882, and 1.491 for F1, F2, and F3, respectively

**Conclusion:** The combination of digitization, laser imaging and machine learning allows for a quantitative analysis of fibrosis on unstained liver biopsy tissue. This methodology can improve the evaluation of NASH candidate treatments and offers advantages to address liabilities of current methods. By capturing and quantifying important morphological features, it is more precise and more amenable to detecting changes. With further validation data, qFibrosis could provide liver biopsy information that are more clinically relevant to support diagnosis and treatment decisions.

**Figure:**

**Three "staining" methods: Chemical (left), laser (middle), and machine-learning (right)**




Chemical staining "Laser staining" "Machine learning staining"

**PT – portal tract; CV – Central vein**

EASL CONGRESS
Vienna, Austria 21–24 June 2023

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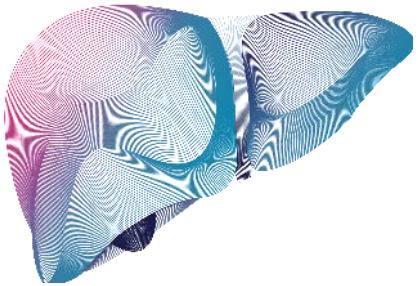
## Digital pathology; Pre-cirrhotic NASH; qFibrosis



## 1 Introduction

Improvement in liver histopathology, especially fibrosis, is a primary outcome measure for Non-Alcoholic SteatoHepatitis (NASH) candidate drugs registration. Besides biopsy size, tissue homogeneity, and staining quality, intra- and inter-observer variability confound the interpretation of current semi-quantitative scores for fibrosis.

## 2 Aim

We describe a novel imaging modality of liver biopsy tissue using stain-free imaging along with Artificial Intelligence (AI) analysis of the digitized image to provide a more sensitive and fully quantitative measurement of fibrosis.

## 3 Methods

- Unstained tissue obtained from liver biopsy is digitized using a proprietary system and submitted to two detection methods (Genesis®200): Second Harmonic Generation (SHG) microscopy to identify collagen and Two-Photon Excitation Fluorescence (TPEF) microscopy to identify hepatocytes.
- Automated Machine Learning (ML) system utilizes SHG/TPEF laser imaging to detect light signals from laser excitation of the biopsy ("laser staining").
- ML then follows the workflow of pathologists, identifying crucial histopathological features like portal tracts, peri-sinusoidal region, peri-portal region, central veins and peri-central region; this is referred to as "Machine learning staining" (**Figure 1**).

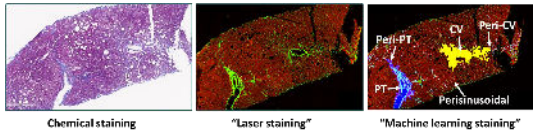



**Figure 1:** Three staining methods: Chemical (left), Laser (middle), Machine-learning (right). PT: Portal Tract, CV: Central Vein

- Specific collagen morphological features such as length, width and area of fibers are then identified by AI (**Figure 2**). Quantification of these parameters are performed based on their distribution patterns in the histopathological regions defined previously.
- Finally, these parameters are combined into an index (qF) by correlating them with pathologists' staging results using NASH-CRN classification system.

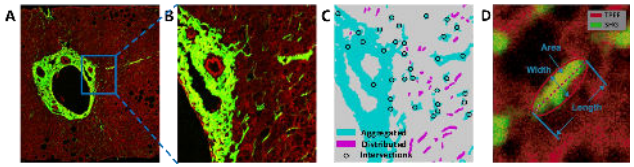



**Figure 2:** Evaluation of collagen parameters (A) SHG/TPEF image; (B) high magnification imaging of area denoted within the blue square in Figure 2A; (C) AI identification of aggregated and distributed strings; (D) Illustration of string length, string area, string width.

## 4 Results

- A total of 17 collagen features (**Table 1**) were selected by ML for qF calculation where the most important features (with largest coefficients) are number of intersections and number of short and aggregated fibers in the whole tissue section.

**Table 1:** 17 collagen features selected by ML for qF calculation

| No. | Features | Description | No. | Features | Description |
|---|---|---|---|---|---|
| 1 | CollagenAreaAll | The area of collagen at overall region | 10 | FiberAWidthDisCV | The total average width of distributed fibers at central vein region |
| 2 | #FiberAll | The number of fibers at overall region | 11 | FiberLengthDisCV | The total length of distributed fibers at central vein region |
| 3 | #ShortFiberAll | The number of short fibers at overall region | 12 | #LongFiberDisCV | The number of long and distributed fibers at central vein region |
| 4 | #ThinFiberAll | The number of thin fibers at overall region | 13 | #ThickFiberDisCV | The number of thick and distributed fibers at central vein region |
| 5 | #IntersectionAll | The number of intersections at overall region | 14 | FiberLengthDisPT | The total length of distributed fibers at portal tract region |
| 6 | #ShortFiberAggAll | The number of short and aggregated fibers at overall region | 15 | #LongFiberDisPT | The number of long and distributed fibers at portal tract region |
| 7 | #ThickFiberAggAll | The number of thick and aggregated fibers at overall region | 16 | FiberAWidthPS | The total average width of fibers at perisinusoidal region |
| 8 | #ThinFiberCV | The number of thin fibers at central vein region | 17 | #LongFiberAggPS | The number of long and aggregated fibers at perisinusoidal region |
| 9 | #ThinFiberAggCV | The number of thin and aggregated fibers at central vein region | | | |

- qF has open-ended ranges, but typical values are between 0 – 5.4 in pre-cirrhotic NASH patients (F0-F3), with higher values representing increasing severity of fibrosis.
- The qF cut-off values correlating with semi-quantitative NASH-CRN scores are **1.04,1.45, and 2.12 for F1, F2, and F3,** respectively. The Spearman's correlation between qF and pathologist fibrosis stage was 0.78 in a non-cirrhotic cohort (**Figure 3**).
- qF has demonstrated good correlation with different pathologists staging in other studies.

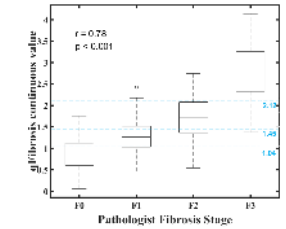



**Figure 3:** Box-Whisker plots showing correlation between qF continuous value and pathologist fibrosis stage for non-cirrhotic cohort

## 5 Conclusions

- This description explains how ML-based AI can be developed by following the workflow of pathologists' in assessing fibrosis in needle biopsies for pre-cirrhotic NASH patients.
- Initial stage involves identifying crucial histopathological features outlined in established classification systems (e.g., NASH-CRN staging) and enabling pathologists to access these AI-generated imaging results.
- Subsequent quantification relies on identified collagen features, facilitating the development of a qF index through correlation with staging results of pathologists.
- Finally, pathologists can visualize "machine learning staining" images and quantification results from qF during fibrosis assessment of liver needle biopsies.

## 6 References


**Sun W, Chang S, Tai D C, et al.** Nonlinear optical microscopy: use of second harmonic generation and two-photon microscopy for automated quantitative liver fibrosis studies. *Journal of Biomedical Optics, 2008, 13(6):7-0.*


## 7 Contact information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