
**BACKGROUND and AIMS**

We have previously shown that the Phenotypic Fibrosis Composite Score (Ph-FCS) calculated by the FibroNest image analysis platform from second harmonic generation (SHG) images correlates with the NASH-CRN fibrosis scores. In this study, the same sections imaged non-destructively by SHG have been stained, digitized and then quantified with FibroNest. We compare performance of the Ph-FCS obtained from these two imaging methods.

**METHOD**

**Tissue Preparation, Instrumentation, and Workflow**

- FFPE sections (~4 microns) of patient liver biopsies (unembedded) are mounted on (no cover slip), deparaffinized and imaged ("SHG image") on the Genesis200™ SHG/2PE microscope from Hamamatsu, at 20X (0.37 micron per pixel). This imaging method is non-destructive.
- The same slides are subsequently stained for collagen using picrosiris red, and imaged at 20X (0.50 micron per pixel) on the Aperio AT Digital Pathology system ("WSI Image").
- Both sets of Digital Images are analyzed and quantified using the FibroNest method:
  - Using Quantitative Image Analysis (FibroNest™) the fibrosis phenotype is described for its collagen content and structure (12 traits), the morphometric traits of the collagen fibers (13 traits), and fibrosis architecture traits (7). In each image, each morphometric and texture trait is represented by a histogram distribution (e.g., Fiber Skeleton Length).
  - The histogram for each trait is described by up to seven quantitative fibrosis parameters (qFPs, 315 in total) to account for mean, variance, distortion and progression.
  - To detect phenotypic differences between the F sub groups, principal qFPs are automatically detected if their group mean value difference is statistically (P<0.05, T-Test) greater than 20%.
  - Principal qFPs are used individually and collectively to describe the differences in phenotypes between groups. They are combined into a normalized Phenotypic Composite Fibrosis Score, a continuous quantifier of the fibrosis phenotype.
  - The composite fibrosis scores are compared to the Pathologist assessment.

**RESULTS**

**Fibrosis Phenotypic Heat Maps and Composite Scores**

For each patient (column), the Fibrosis Phenotypic map (above) visualizes the relative severity (green to red) of the quantitative fibrosis traits (qFPs) as quantified from the image, and automatically selected to account for variability between groups. Each row represents a quantifiable trait. The normalized quantitative traits values are combined to generate a phenotypic Fibrosis composite score for each patient. The phenotypic map and augmented images can be used to assist pathologists for staging and reduce the Categorical Staging Inaccuracy.

**Conclusions**

- The phenotype of Fibrosis in NASH does not depend on the staining and imaging method and is well quantified by FibroNest. The FibroNest composite scores correlate well with NASH-CRN stages and can be tailored to answer specific phenotypic questions in NASH.