

# Is the histological phenotype of Fibrosis different between LEAN and OBESE NASH patients?

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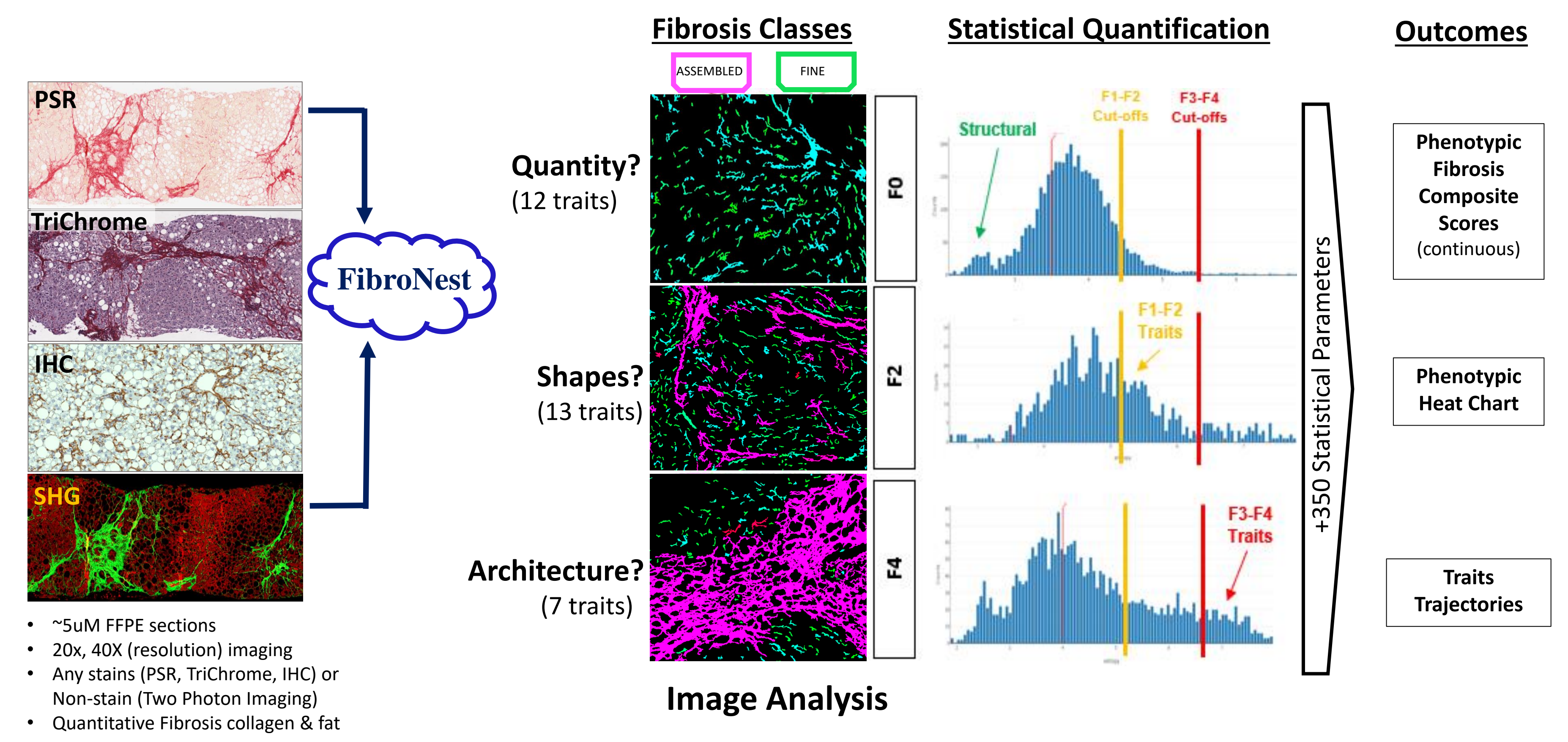
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## BACKGROUND and AIMS

Although nonalcoholic fatty liver disease (NAFLD) is commonly associated with obesity, non-obese patients can also develop NAFLD (“Lean NAFLD”). Little is known about the possible subtle histological differences between these two sub phenotypes, in particular for fibrosis. Here, we used quantitative Digital Pathology image analysis to investigate the differences of the histological phenotype of fibrosis between Lean and Obese nonalcoholic steatohepatitis (NASH) patients with F2-3 fibrosis.

## METHOD

### TISSUE PREPARATION, INSTRUMENTATION, AND WORKFLOW

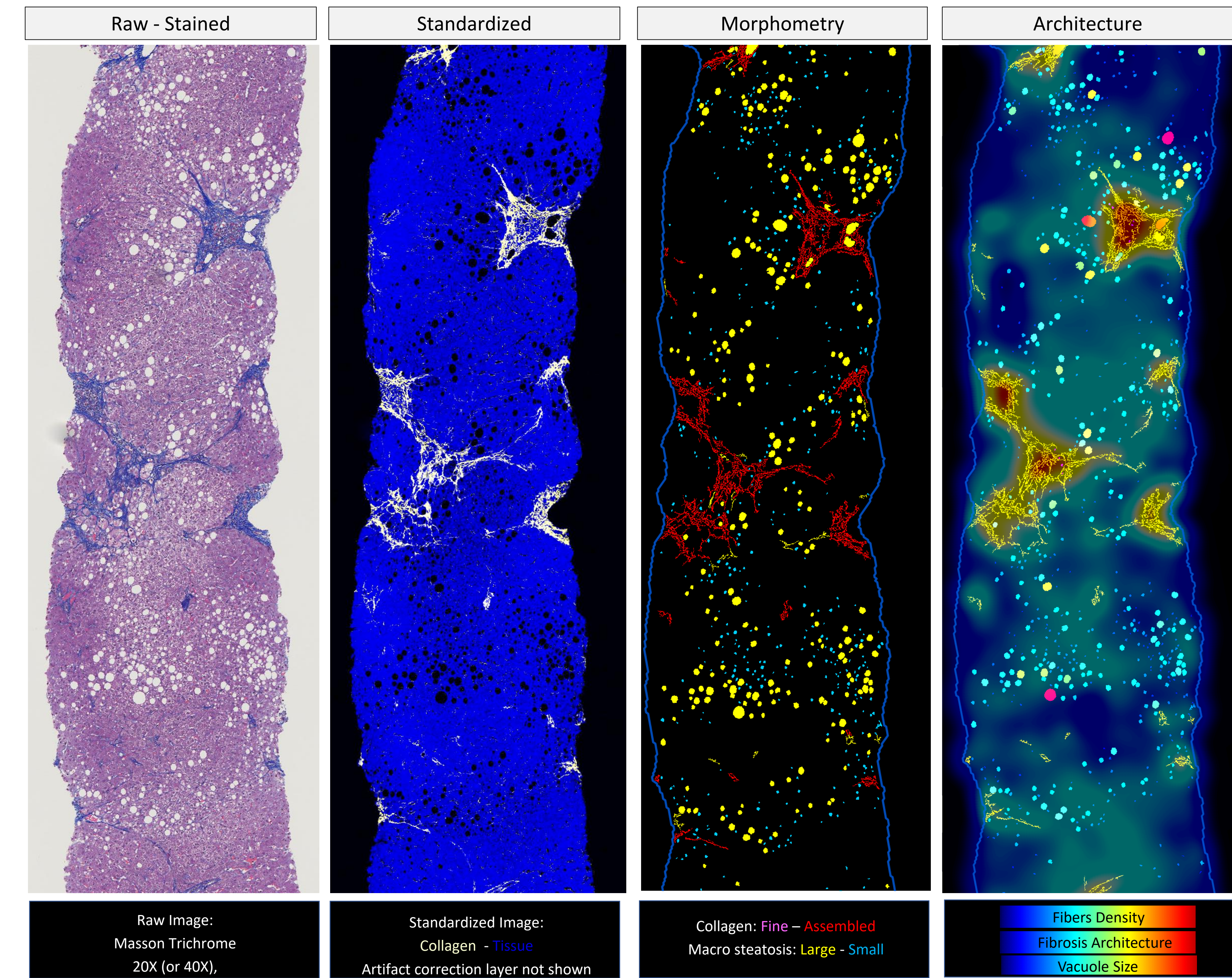


Group	Description	Histological Assessment	Total N 35
NAFLD LEAN	NAFLD Patients with BMI<25 With F2 (N=9) and F3 (N=8) fibrosis Stage	Histologic assessment and Fibrosis severity stage was assessed by pathologists	N= 19
NAFLD OBESE	NAFLD Patients with BMI<30 With F2 (N=10) and F3 (N=8) fibrosis Stage		

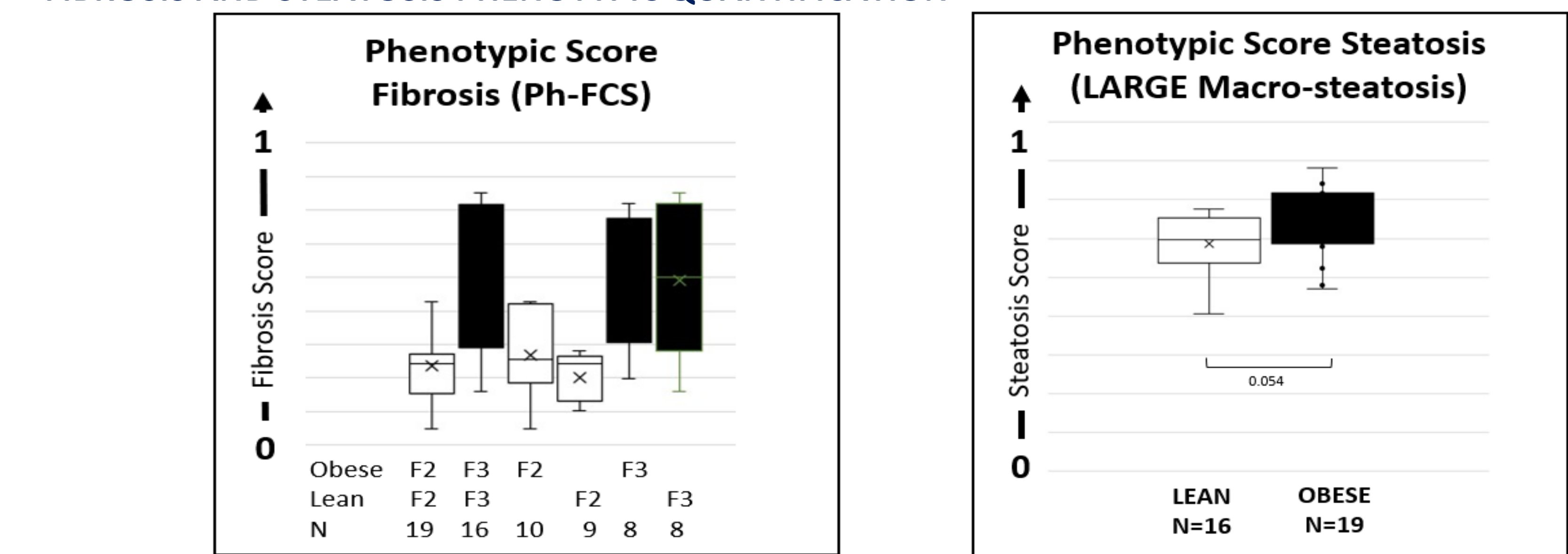
- FFPE sections (~4 microns) of patient liver biopsies were deparaffinized, stained with Masson Trichrome for Collagen and digitized at 20X (0.44 micron/pixel) on a Hamamatsu WSI system
- Digital Biopsy Adequacy: Five digital biopsies from the initial cohort of 40 patients were excluded due to non adequacy (poor processing, staining or scanning)
- Using 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 two 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 (Ph-CFS), a continuous quantifier of the fibrosis phenotype.

## RESULTS

### REPRESENTATIVE IMAGES AND FIBRONEST ANALYSES

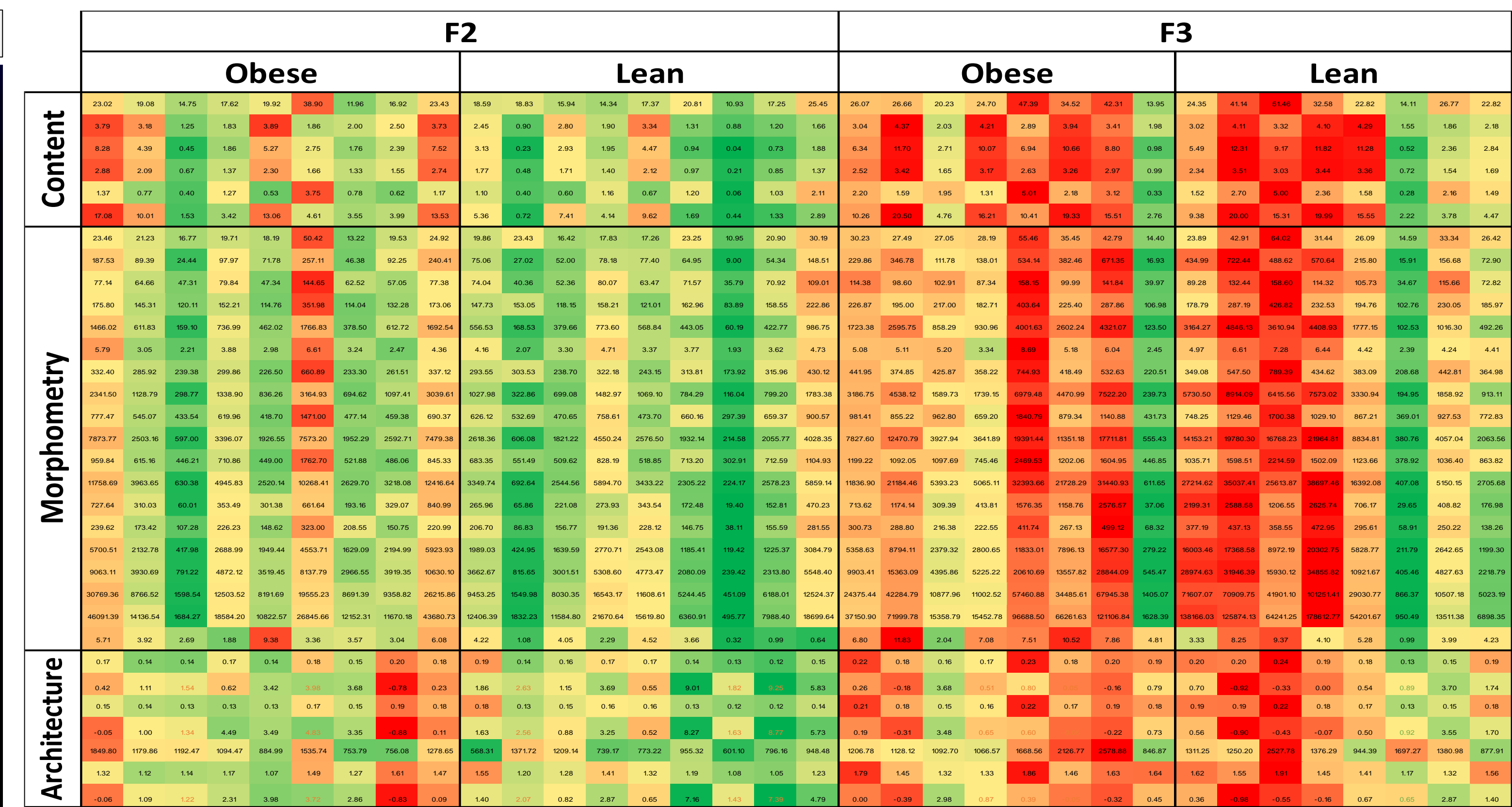


### FIBROSIS AND STEATOSIS PHENOTYPIC QUANTIFICATION



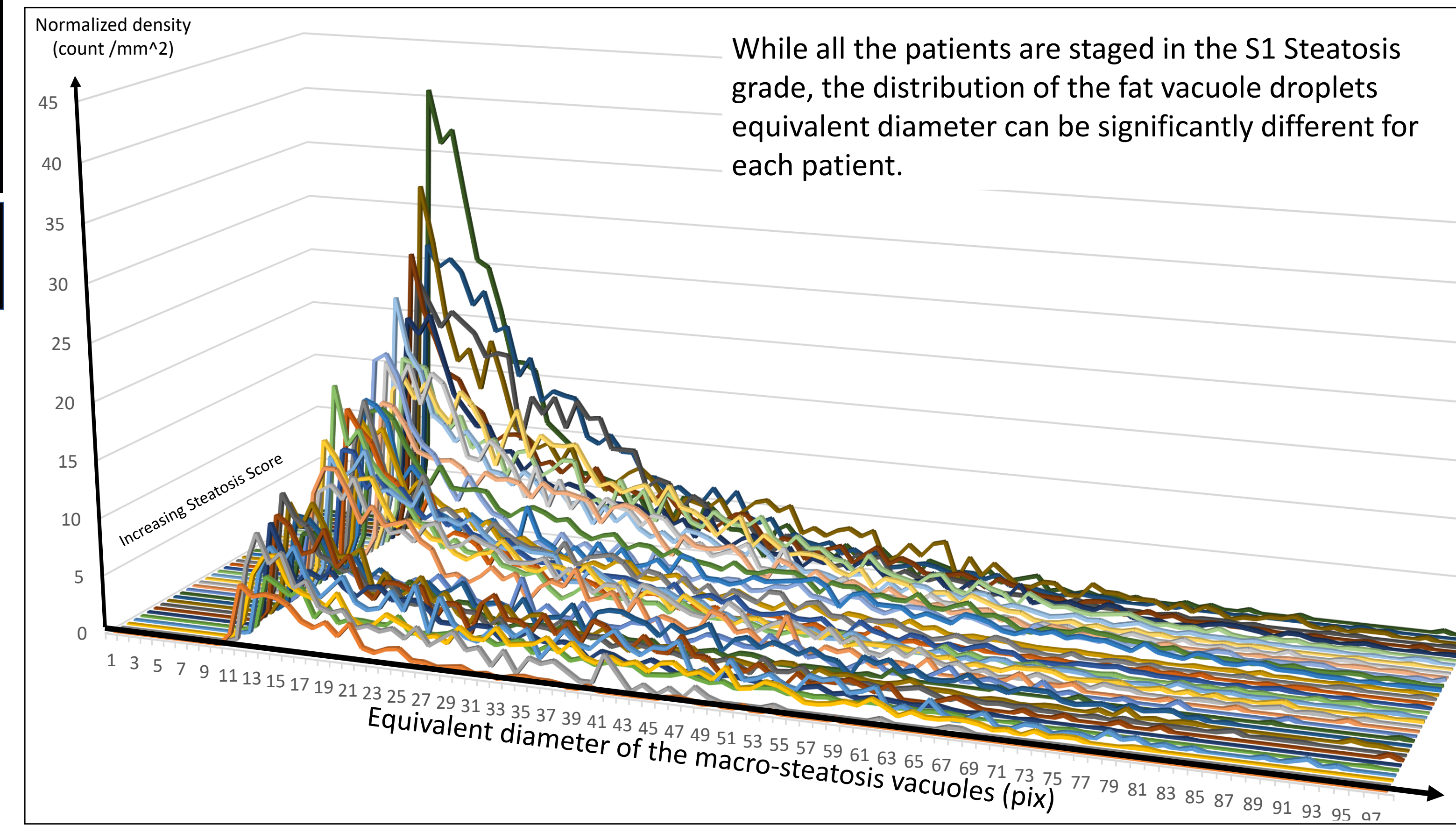
The Ph-FCS was consistent with the NASH-CRN stage for each group (left chart), we did not find statistically significant differences between the Lean and Obese groups at any of phenotypic layers explored by FibroNest. In aggregate, the fibrosis phenotypic scores were the same in the Lean and Obese NASH. Subtle differences are observed (right chart) in the demographics of large macro-vesicular steatosis, consistent with the phenotype of obese patients.

### FIBROSIS PHENOTYPIC HEAT MAPS (EACH ROW IS A PRINCIPAL PHENOTYPIC TRAIT)



For each patient (column) the Fibrosis Phenotypic maps (above) visualizes the relative severity (green to red) of the quantitative fibrosis traits (qFTs) as quantified from the image, and automatically selected to account for variability between groups. The phenotypic map can be used to QC the pathologists staging. The normalized quantitative traits values are combined to generate a phenotypic Fibrosis composite score. The similar concept can be used for Steatosis phenotype (including % steatosis, and mean, median, standard distribution, skewness and kurtosis fat vacuole size distribution, illustrated below).

### STEATOSIS PROFILES (FAT VACUOLES SIZE DISTRIBUTIONS)



## Conclusion

Sophisticated quantitative Digital Pathology Image analysis did not detect differences in the histological phenotype of fibrosis between the Lean and Obese patients.