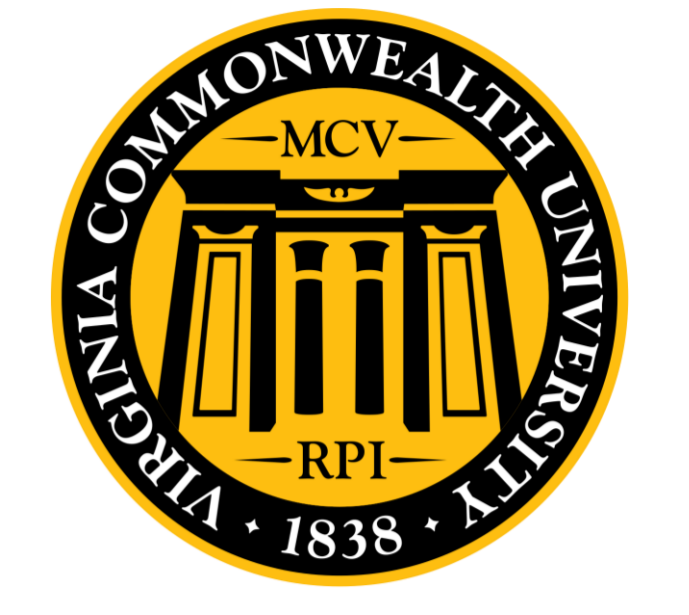


Description of Translational Fibrosis Phenotypes between the 3D NASH Spheroid Model and Human NASH



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1 Introduction

The adoption of spheroid models in the screening of anti-fibrotic compounds for Non-Alcoholic Steatohepatitis (NASH) has led to the need to understand the phenotypic relevance of the fibrosis histological phenotypes and their clinical translational relevance.

2 Aim

Here, we use a novel Digital Pathology quantitative image analysis and AI platform, FibroNest™, to generate and measure quantifiable Fibrosis Traits (qFTs) in Human in-vitro 3D NASH tissue models and natural cohort of patients diagnosed with NASH. Hundreds of histological parameters are measured in both models and a Venn diagram approach is used to identify those traits that describe histological fibrosis severity in both models.

3 Method

- Two 3D-NASH spheroid-colony groups (**Lean n=17, NASH n=14**)
 - 3D liver tissues were either treated for 10 days with free fatty acids and LPS or not to generate NASH and lean conditions respectively
- A retrospective clinical cohort (**n=104** patients) with NASH diagnosed by histologic assessment of liver biopsy according to NASH CRN criteria by pathologists (**F0(26), F1(24), F2(28), F3(20), F4(6)**).

Spheroid Information

- Human in vitro 3D InSight™ liver microtissues
- Contain primary hepatocytes, Kupffer cells, endothelial cells and hepatic stellate cells

Pathology, Digital Pathology and AI:

- 4µm thick Spheroid FFPE slices were stained with **Picro Sirius Red** and scanned at **40x**
- FFPE sections of liver human biopsies were stained with **Picro Sirius Red** and scanned at **20x**
- Scanning** was done with bright-light whole slide imaging scanners.
- Fibrosis severity continuous score (Ph-FCS, 1 to 10)**. Quantitative image analysis extracts single-fiber quantitative traits (qFTs, N=315) from the fibrosis histological phenotype. Principal qFTs are automatically detected to best describe the progression of the fibrosis in both models and combined into a normalized Phenotypic Composite Fibrosis Score (Ph-FCS).
- A **Venn diagram** approach was used to identify those traits that describe histological fibrosis severity in both models. qFTs are normalized to their initial value and their folds describe relative levels of progression which can be benchmarked from a model to another
- Additional **sub-Phenotypic scores** (fine and assemble fiber sub-classes, morphometry, architecture, fibrosis scar) are used to further describe the fibrosis phenotypes and its remodeling as fibrosis progress or regresses

5 Conclusions

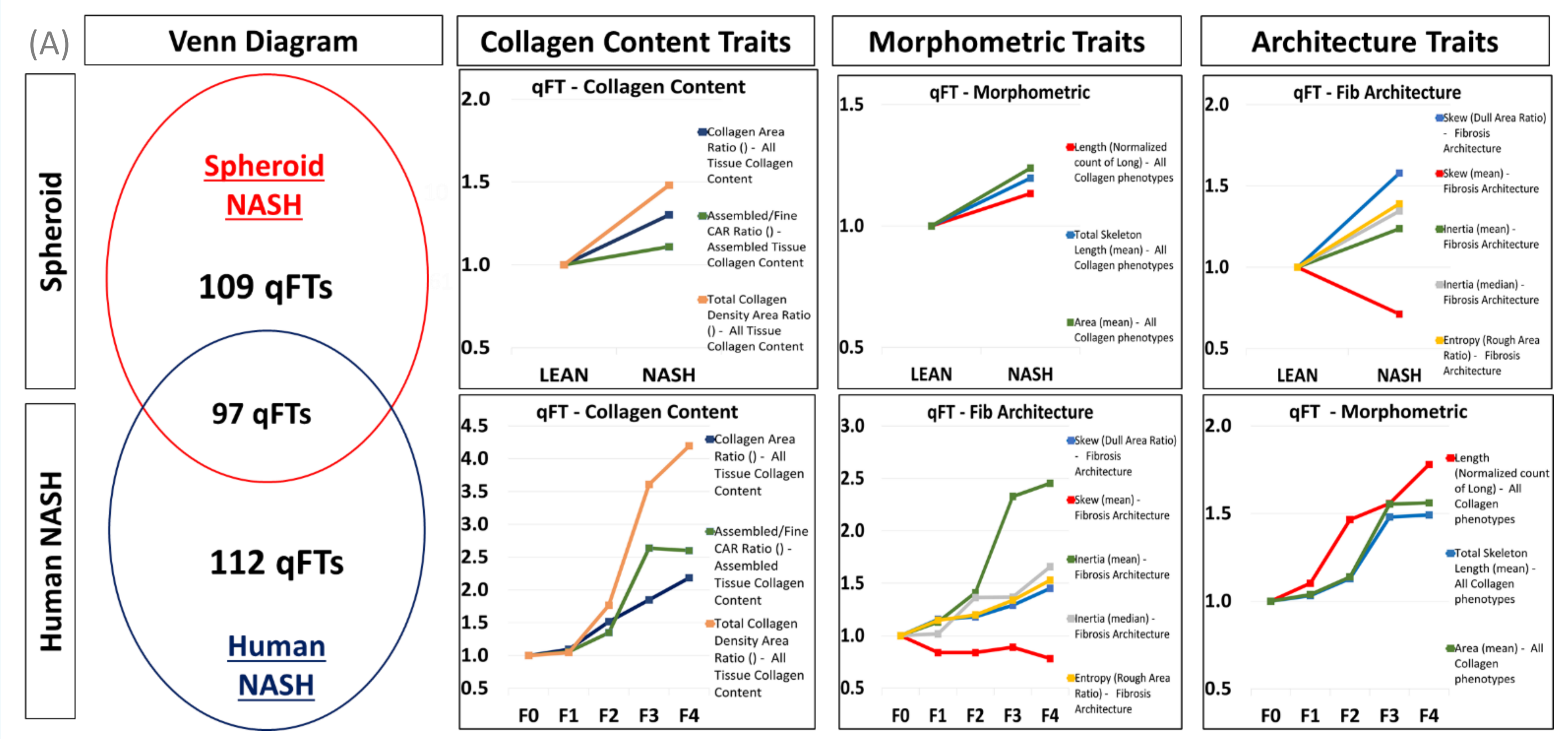
We identified 97 histological traits of fibrosis severity phenotype that can be translated from the human 3D NASH spheroid model to clinical F2 or F3 NASH CRN stages. These traits will be used to evaluate the anti-fibrotic compounds effect in 3D NASH model to predict their effect in human.

6 Contact information

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4 Results

- The Venn Diagram identifies 97 traits shared between the two fibrosis progression models (B).



- 10 traits** described high-level collagen features such as Collagen Area Ratio, Assembled/Fine Collagen fibers area ratio, and density (intensity weighted) area ratio (selected qFTs on figure A).
- 61 traits** described common morphometric features, such as the proportion of collagen fibers that are long, the mean fiber skeleton length, and the mean fiber area (selected qFTs on figure A).
- 26 traits** described common architectural features (selected qFTs on figure A).
- The spheroid NASH model cannot be directly associated to a specific human NASH CRN stage number. However, specific qFTs in the NASH spheroid model can be associated with either F2 or F3 stages based on their fold-change values.

(B)

Collagen Content	qFT Full Name	colMod
	Fiber Normalized Count (I) - All Tissue Collagen Content	1
	Skeleton Nodes Normalized Count (I) - All Tissue Collagen Content	1
	Skeleton Branch Normalized Count (I) - All Tissue Collagen Content	1
	Collagen Area Ratio (I) - All Tissue Collagen Content	1
	Collagen Area Ratio (SQRT) - All Tissue Collagen Content	1
	Collagen Area Ratio (I) - Assembled Tissue Collagen Content	1
	Collagen Area Ratio (SQRT) - Assembled Tissue Collagen Content	1
	Assembled/Fine CAR Ratio (I) - Assembled Tissue Collagen Content	1
	Total Collagen Density Area Ratio (I) - All Tissue Collagen Content	1
	Branches (Normalized count of Assembled) - All Collagen phenotypes	1
	Length (mean) - All Collagen phenotypes	1
	Length (std) - Assembled Collagen phenotypes	1
	Length (skew) - Assembled Collagen phenotypes	1
	Length (kurtosis) - Assembled Collagen phenotypes	1
	Length (mean) - All Collagen phenotypes	1
	Length (std) - All Collagen phenotypes	1
	Length (skew) - All Collagen phenotypes	1
	Length (kurtosis) - All Collagen phenotypes	1
	Total Skeleton Length (std) - All Collagen phenotypes	1
	Total Skeleton Length (skew) - Assembled Collagen phenotypes	1
	Total Skeleton Length (kurtosis) - Assembled Collagen phenotypes	1
	Width (Normalized count of Thick) - Assembled Collagen phenotypes	1
	Width (skew) - Assembled Collagen phenotypes	1
	Width (kurtosis) - Assembled Collagen phenotypes	1
	Perimeter (mean) - All Collagen phenotypes	1
	Perimeter (std) - All Collagen phenotypes	1
	Perimeter (skew) - Assembled Collagen phenotypes	1
	Perimeter (kurtosis) - Assembled Collagen phenotypes	1
	Area (mean) - All Collagen phenotypes	1
	Area (std) - All Collagen phenotypes	1
	Area (skew) - Assembled Collagen phenotypes	1
	Area (kurtosis) - Assembled Collagen phenotypes	1
	Perimeter (Normalized count of Tortuosity) - All Collagen phenotypes	1
	Tortuosity (Normalized count of Tortuosity) - All Collagen phenotypes	1
	Eccentricity (Normalized count of Elongated) - All Collagen phenotypes	1
	Solidity (Normalized count of Compact) - All Collagen phenotypes	1
	Orientation (Normalized count of Vertical) - All Collagen phenotypes	1
	Length (Normalized count of Long) - Fine Collagen phenotypes	1
	Length (std) - Fine Collagen phenotypes	1
	Total Skeleton Length (std) - Fine Collagen phenotypes	1
	Width (skew) - Fine Collagen phenotypes	1
	Perimeter (std) - Fine Collagen phenotypes	1
	Area (std) - Fine Collagen phenotypes	1
	Area to Perimeter Ratio (kurtosis) - Fine Collagen phenotypes	1
	Solidity (skew) - Fine Collagen phenotypes	1
	Orientation (Normalized count of Vertical) - Fine Collagen phenotypes	1
	Branches (Normalized count of Assembled) - Assembled Collagen phenotypes	1
	Branches (mean) - Assembled Collagen phenotypes	1
	Branches (std) - Assembled Collagen phenotypes	1
	Branches (skew) - Assembled Collagen phenotypes	1
	Branches (kurtosis) - Assembled Collagen phenotypes	1
	Length (Normalized count of Long) - Assembled Collagen phenotypes	1
	Length (mean) - Assembled Collagen phenotypes	1
	Length (std) - Assembled Collagen phenotypes	1
	Length (skew) - Assembled Collagen phenotypes	1
	Length (kurtosis) - Assembled Collagen phenotypes	1
	Total Skeleton Length (mean) - Assembled Collagen phenotypes	1
	Total Skeleton Length (std) - Assembled Collagen phenotypes	1
	Total Skeleton Length (skew) - Assembled Collagen phenotypes	1
	Total Skeleton Length (kurtosis) - Assembled Collagen phenotypes	1
	Width (Normalized count of Thick) - Assembled Collagen phenotypes	1
	Width (skew) - Assembled Collagen phenotypes	1
	Width (kurtosis) - Assembled Collagen phenotypes	1
	Perimeter (mean) - Assembled Collagen phenotypes	1
	Perimeter (std) - Assembled Collagen phenotypes	1
	Perimeter (skew) - Assembled Collagen phenotypes	1
	Perimeter (kurtosis) - Assembled Collagen phenotypes	1
	Area (mean) - Assembled Collagen phenotypes	1
	Area (std) - Assembled Collagen phenotypes	1
	Area (skew) - Assembled Collagen phenotypes	1
	Area (kurtosis) - Assembled Collagen phenotypes	1
	Area to Perimeter Ratio (kurtosis) - Assembled Collagen phenotypes	1
	Tortuosity (Normalized count of Tortuosity) - Assembled Collagen phenotypes	1
	Tortuosity (std) - Assembled Collagen phenotypes	1
	Tortuosity (skew) - Assembled Collagen phenotypes	1
	Tortuosity (kurtosis) - Assembled Collagen phenotypes	1
	Eccentricity (skew) - Assembled Collagen phenotypes	1
	Orientation (Normalized count of Vertical) - Assembled Collagen phenotypes	1
	Skew (Dull Area Ratio) - Fibrosis Architecture	1
	Skew (Sharp Area Ratio) - Fibrosis Architecture	1
	Skew (mean) - Fibrosis Architecture	1
	Skew (median) - Fibrosis Architecture	1
	Skew (skew) - Fibrosis Architecture	1
	Skew (kurtosis) - Fibrosis Architecture	1
	Kurtosis (Fraggy Area Ratio) - Fibrosis Architecture	1
	Kurtosis (Crisp Area Ratio) - Fibrosis Architecture	1
	Kurtosis (median) - Fibrosis Architecture	1
	Kurtosis (skew) - Fibrosis Architecture	1
	Kurtosis (kurtosis) - Fibrosis Architecture	1
	Entropy (Binary Area Ratio) - Fibrosis Architecture	1
	Entropy (mean) - Fibrosis Architecture	1
	Entropy (median) - Fibrosis Architecture	1
	Entropy (skew) - Fibrosis Architecture	1
	Entropy (kurtosis) - Fibrosis Architecture	1
	Entropy (Rough Area Ratio) - Fibrosis Architecture	1
	Entropy (mean) - Fibrosis Architecture	1
	Entropy (median) - Fibrosis Architecture	1