



FibroNest: Single-fiber, Single Cell High Content High Resolution Digital Pathology and AI

Liver forum #15 Paris, Septembre 2023

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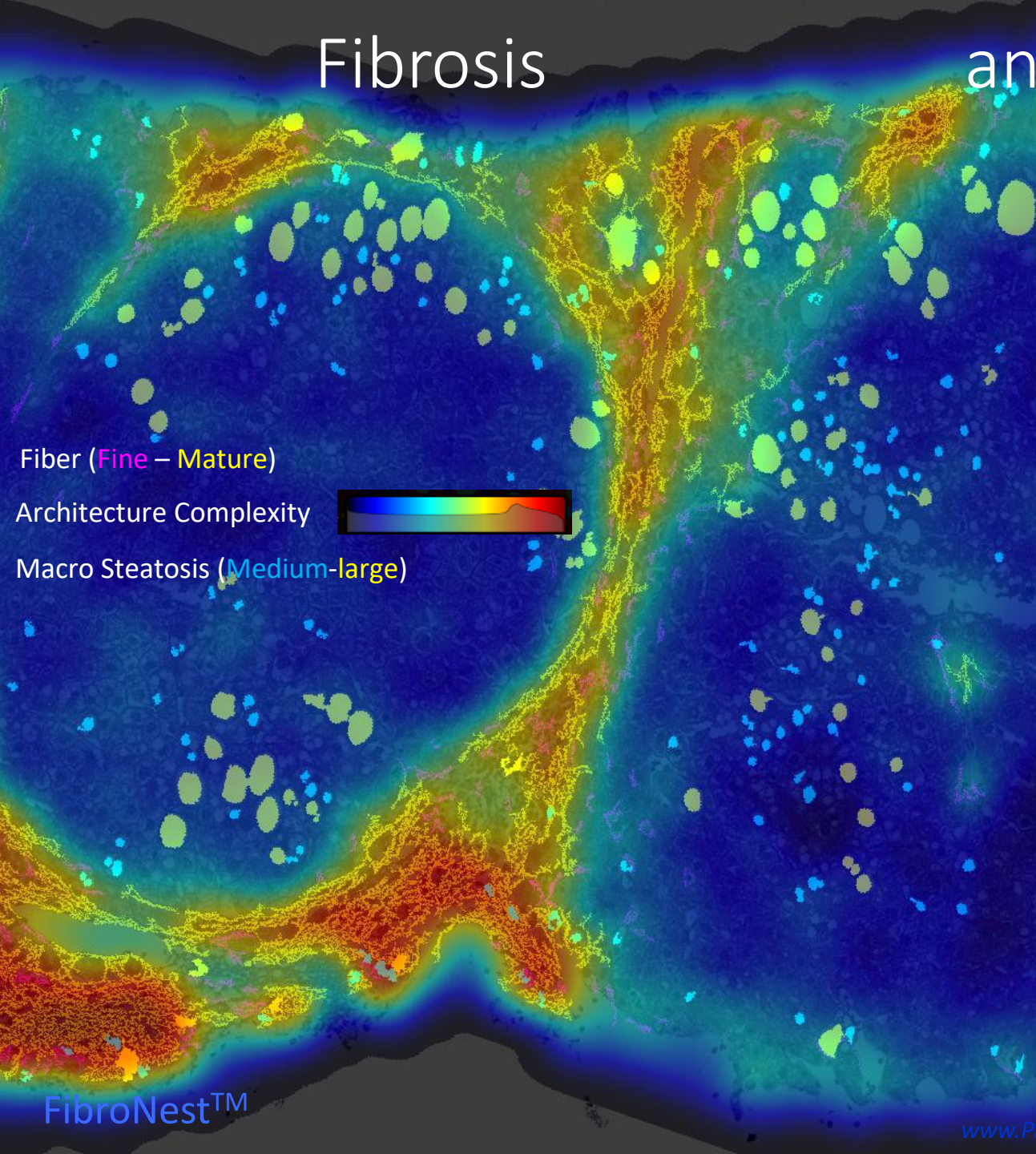
About the FibroNest method

- ❑ **Same Slide(s) as Pathologist**
- ❑ **High Resolution**
- ❑ **High content | Single Fiber | Single Cell Image Analysis**
- ❑ **AI > large quantitative & relevant data-lakes**
- ❑ **Robust vs Pre-Analytical condition**
- ❑ **Fully translational, not trained on existing paradigms**
- ❑ **7.3k Clinical Images, 12k Images**
- ❑ **3 Phase 3, 7 Phase 2 NASH, 3 Phase 2 in other conditions, 60+ preclinical**

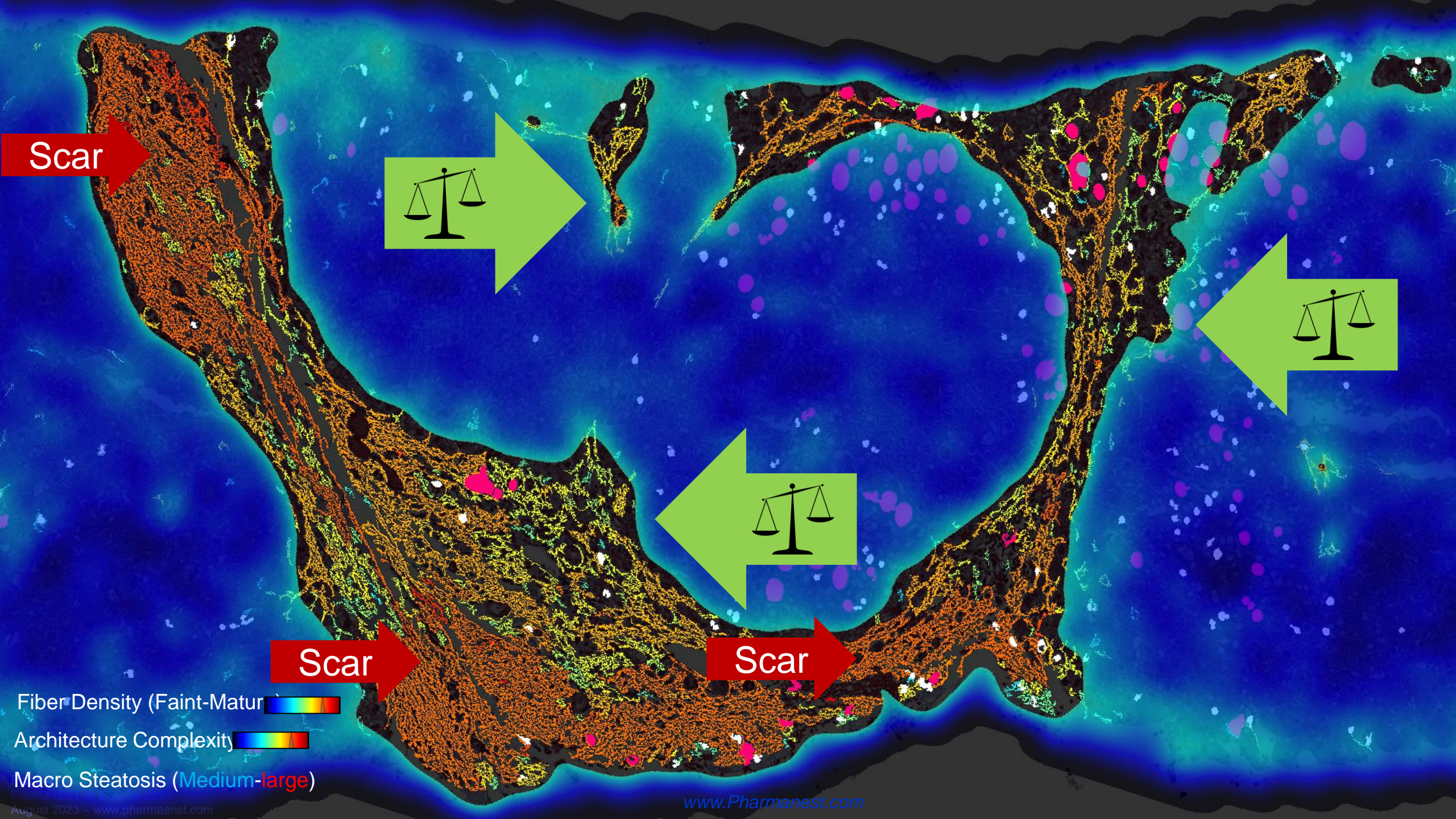
Fibrosis

and

Inflammation



- Normal Hepatocytes
- Steatosis Hepatocytes
- Inflammatory Cells
- Specialized Cells
- Q4-2024
- Stellate Cells
- FibroBlasts
- Ballooned Cells
- LATER
- Glycogenotic Nuclei
- Acidophilic Bodies
- Megamitochondria




Scar

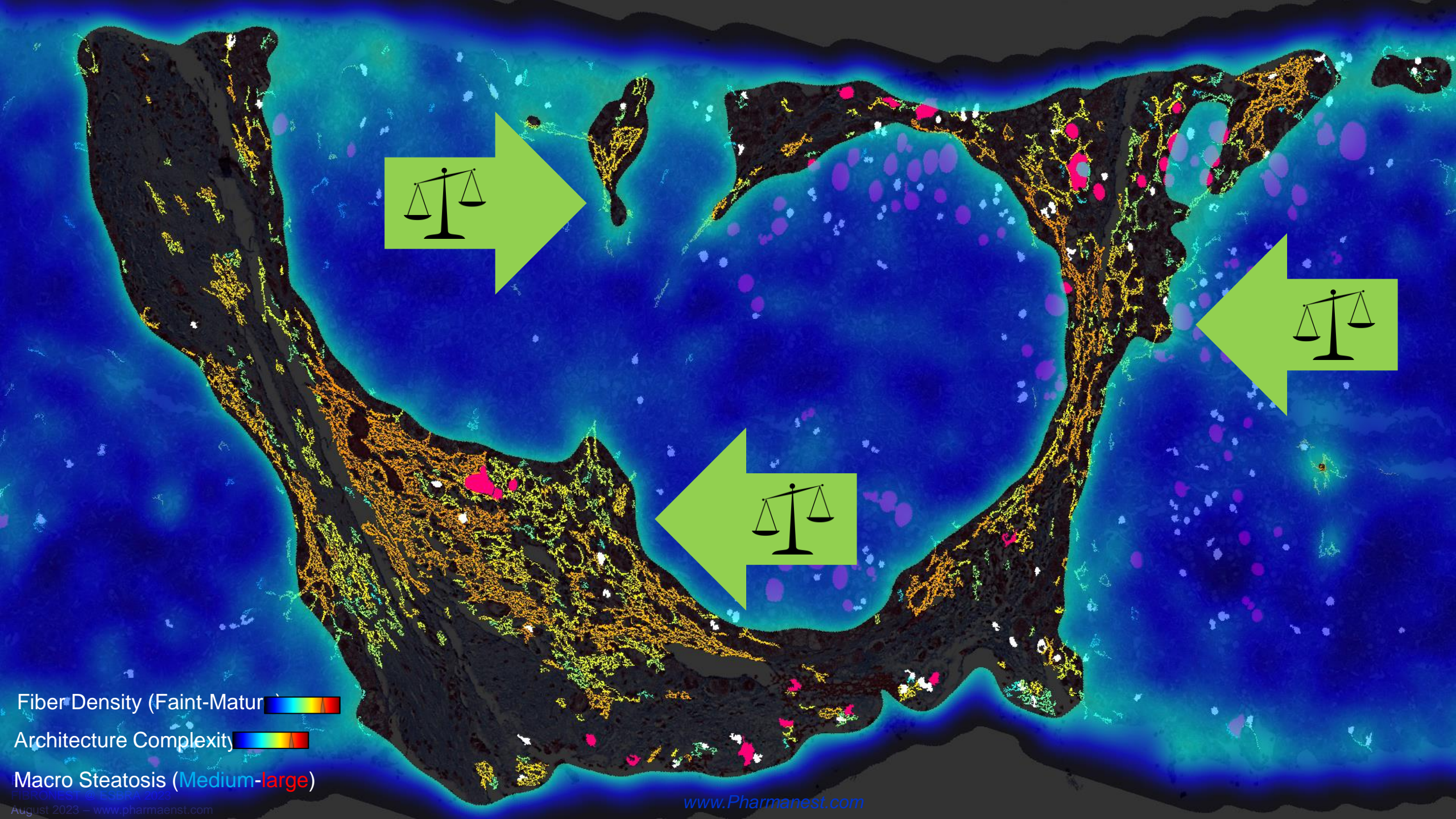
Scar

Scar

Fiber Density (Faint-Matur 

Architecture Complexity 

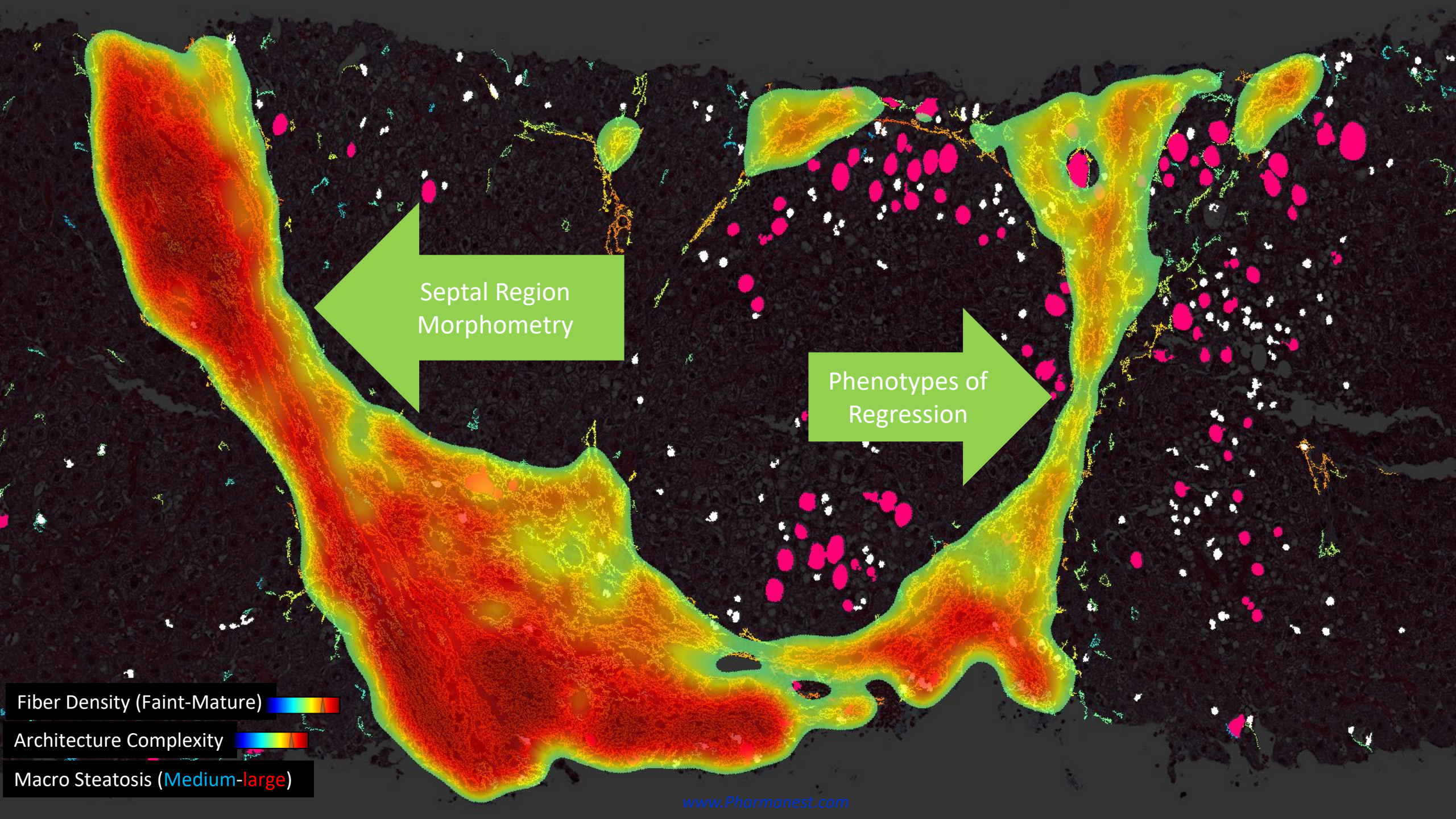
Macro Steatosis (Medium-large)



Fiber Density (Faint-Matur

Architecture Complexity

Macro Steatosis (Medium-large)



FibroNest Analytical Method

Analytical Method Hypothesis and Biological Relevance

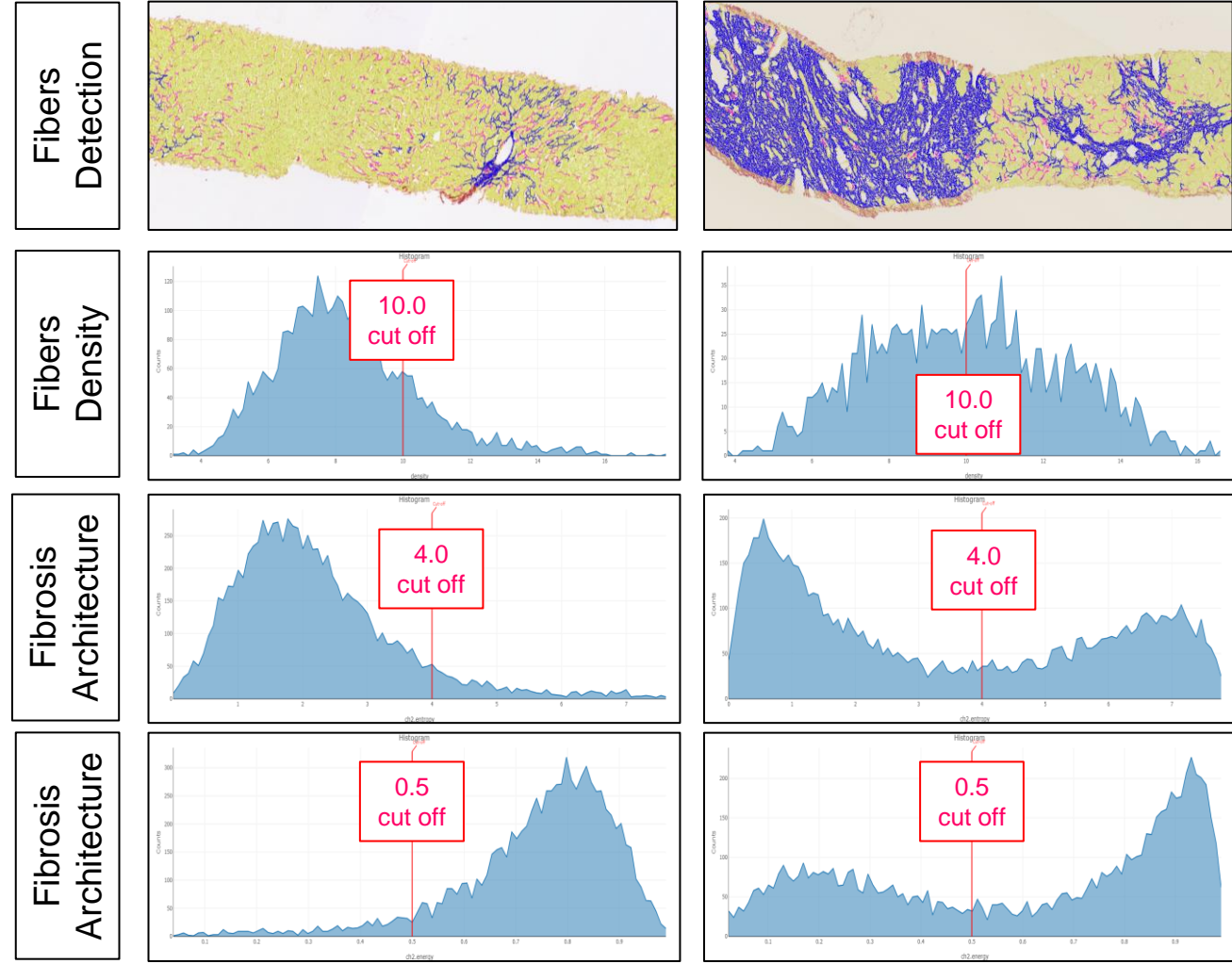
Fibrosis expresses different histological phenotypes.

This histological phenotype is different depending on etiology, severity, prognostic, organs and possibly patient sub-phenotypes

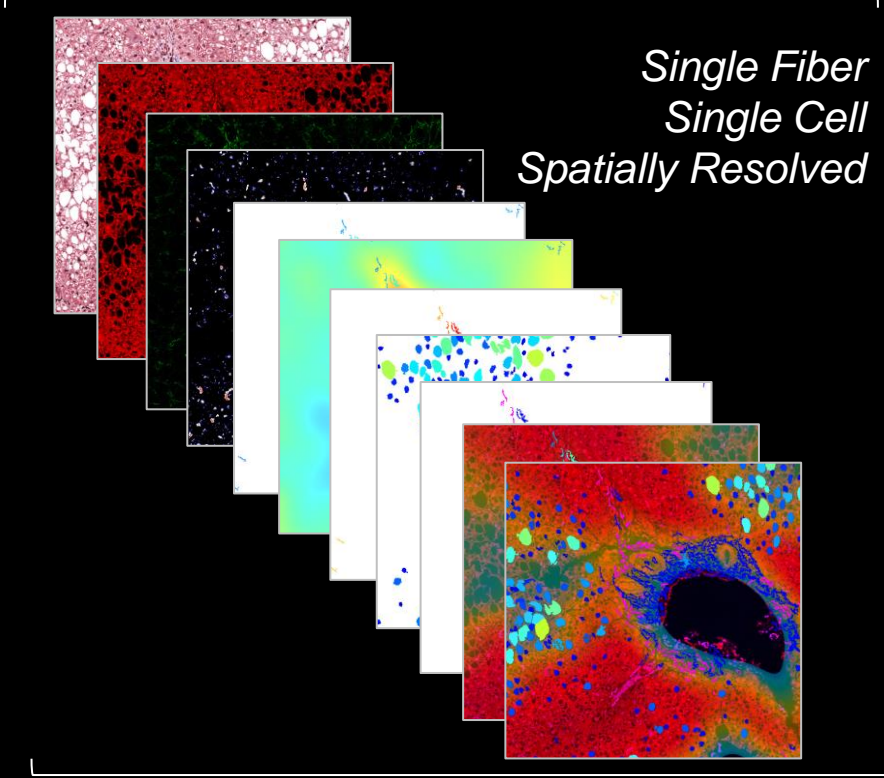
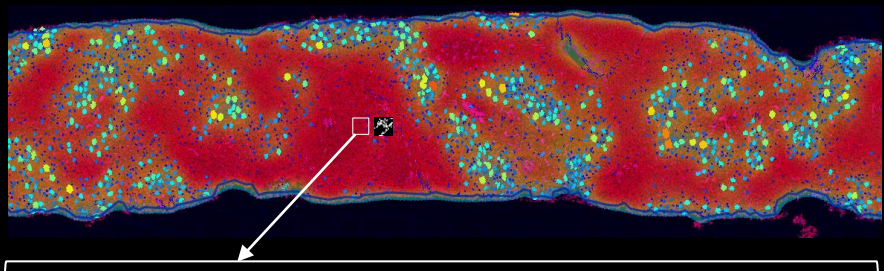
Differences of phenotypes are represented by changes in statistical distributions of its traits in a tissue.

... Does not contradict Histological paradigms

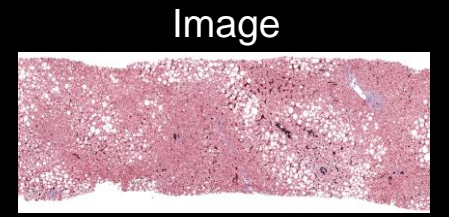
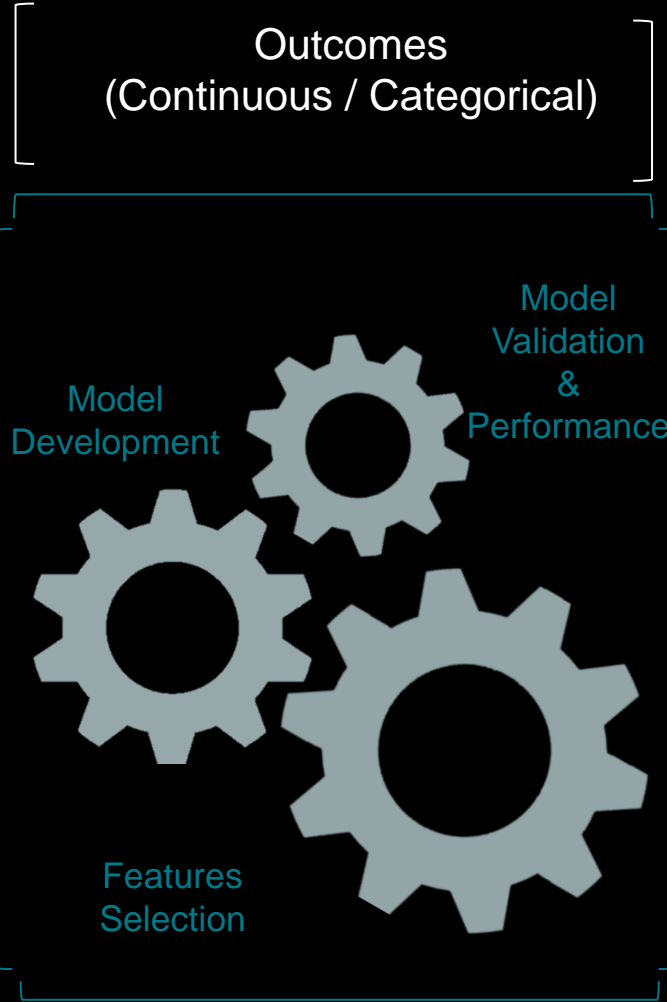
... introduces truly quantitative capabilities



FibroNest Predictive Engine Overview



~5,000 tiles X 3 image channels x 130 data Layers
~6000 fibers X 50 data layers

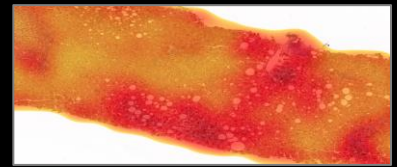


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Composite Scores

Insights on key features

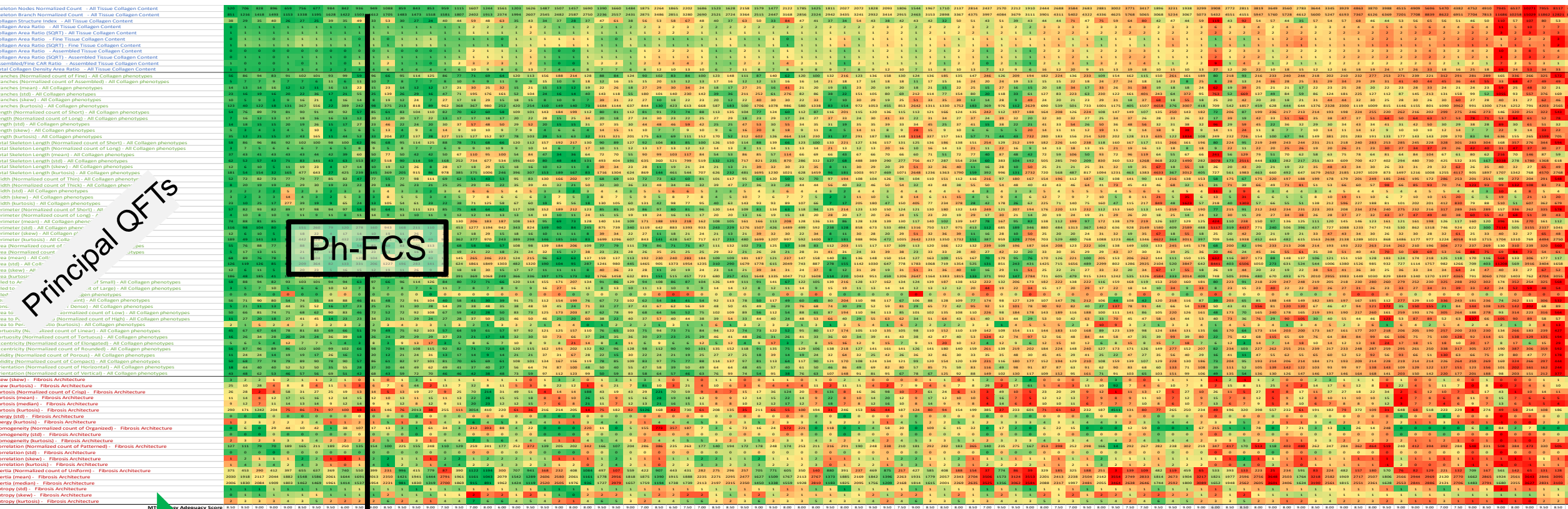
Augmented Pathology



Ph-Nest Analytical Method

Generation of Phenotypic Composite Scores

Increasing Ph-FCS Fibrosis Severity Score



Ph-FCS is a continuous phenotypic biomarker to quantify the severity of Fibrosis in NASH

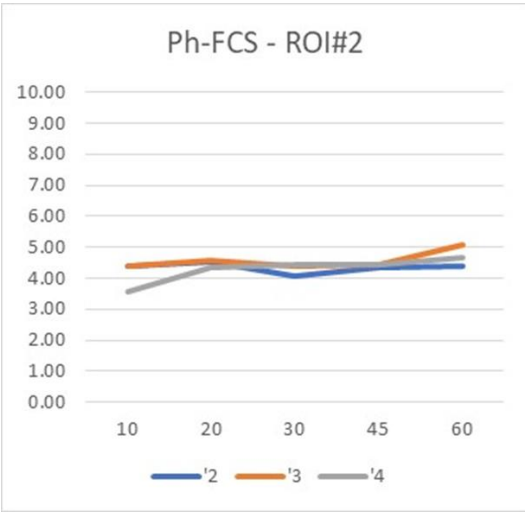
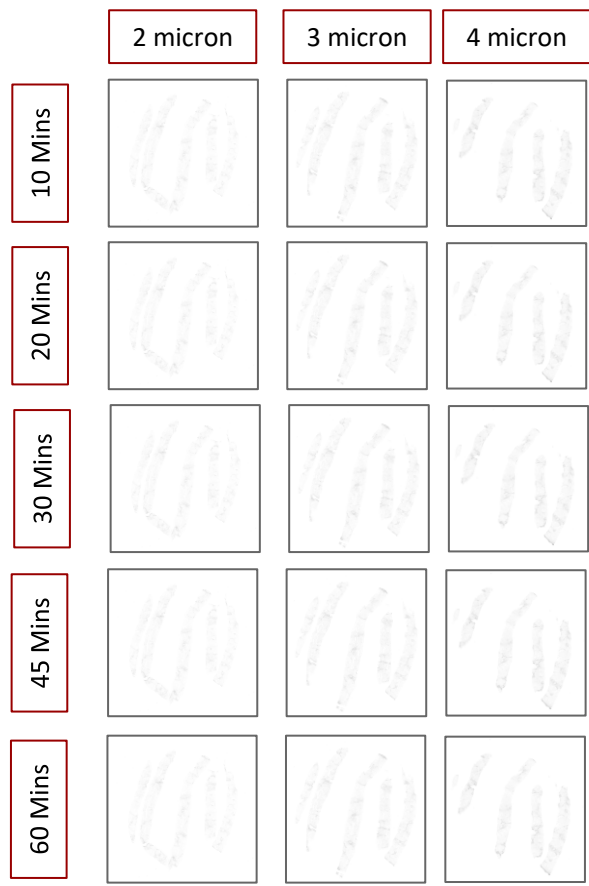
Multimodality assessment of hepatic fibrosis: Ranked paired reading and artificial intelligence identifies fibrosis improvement with Aramchol missed by conventional staging. V. Ratziu, Y. Yilmaz, D. Lazas, S.L. Friedman, C. Lackner, C. Behling, OW. Cummings, Li Chen, M. Petitjean, Y. Gilgun-Sherk, S. Kadosh, and A. J. Sanyal (EALS 2022, [poster here](#))

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 FIBRONEST @ ESBRA 2023
 August 2023 – www.pharmaenst.com

Ph-FCS Fibrosis Biomarker

Current Biomarker Analytical Validation Results

Sensitivity to Pre-analytical Conditions (excl/WSI)

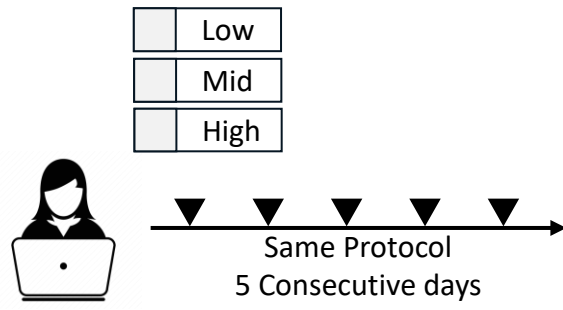


If Bath Length : 20 to 30 mins
 If Tissue Thickness : 3 to 4 microns

Ph-FCS varies from 1% to 6%

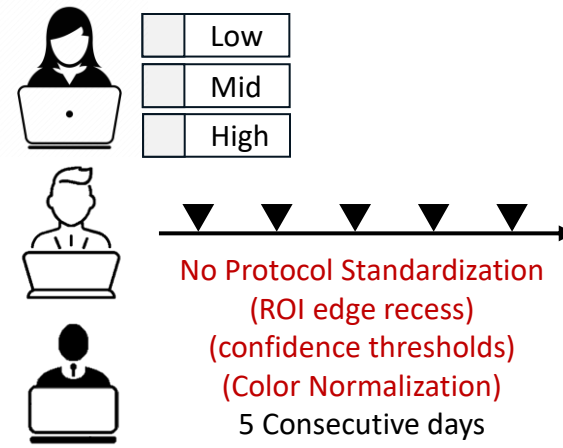
Precision

Repeatability



Coefficient of Variation % (Std/Mean)			
	Low	Mid	High
Pt-Steatosis %	0.57%	0.79%	0.86%
Ph-FCS	0.47%	0.21%	0.29%

Reproducibility

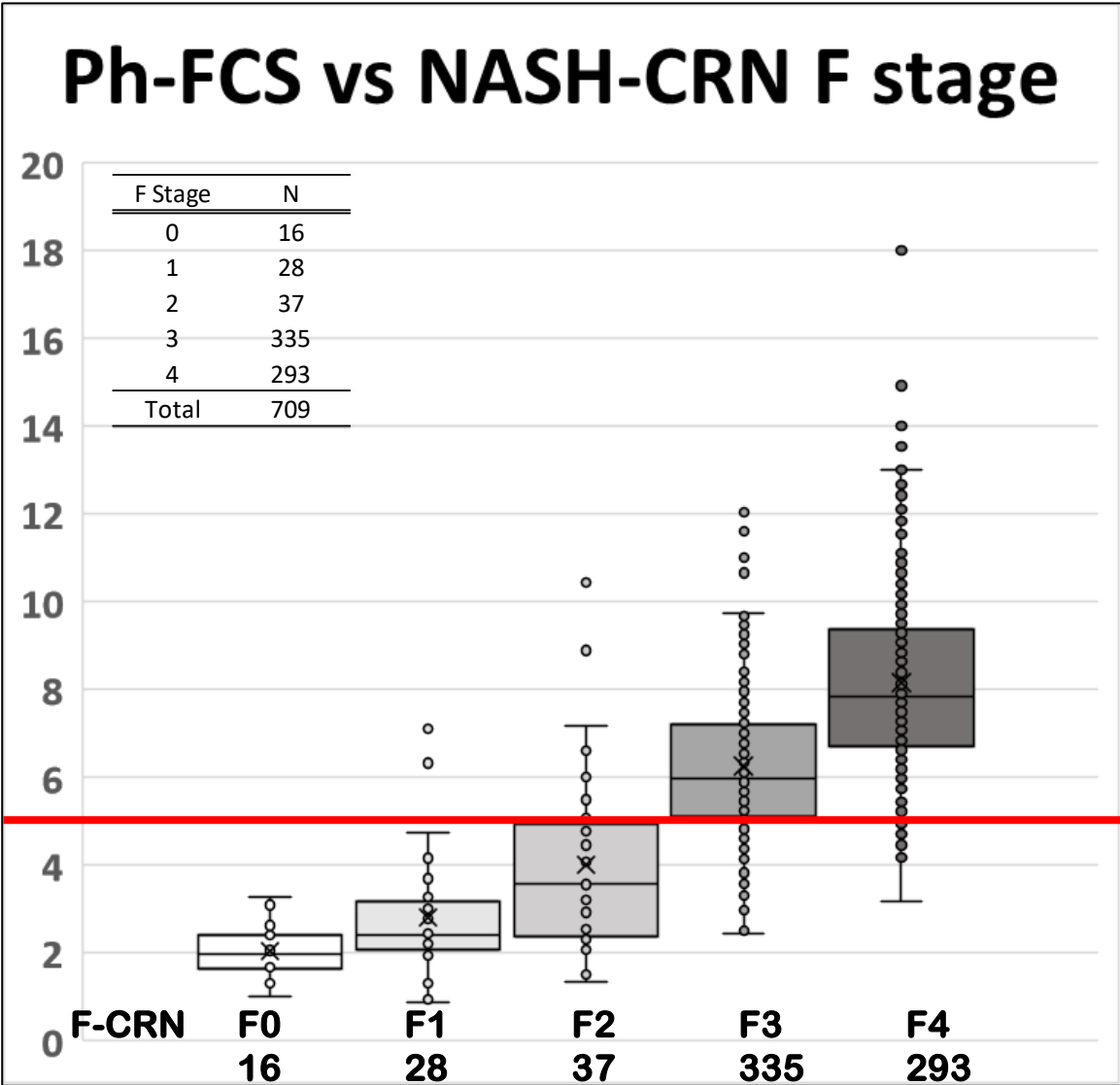


Coefficient of Variation % (Std/Mean)			
	Low	Mid	High
PT-Steatosis %	1.19%	0.74%	2.13%
Ph-FCS	11.53%	4.95%	3.33%

Evaluation of a novel histology-based fibrosis phenotypic composite score and its correlation with NASH-CRN Fibrosis scores in patients with NASH. Li Chen (1), Michael Lung (2), Cynthia Behling (2), Arun Sanyal (3), Mathieu Petitjean (1). 1 - PharmaNest, Princeton, NJ, USA; 2 - University of California, San Diego, NAFLD Research Center, Division of Gastroenterology. 3-Virginia Commonwealth University, Richmond, VA, USA.

Ph-FCS Fibrosis Biomarker

Current Biomarker Clinical performance



Ph-FCS ability to classify based on diagnostic performance (sensitivity, specificity):

- ICD10-K74.01: Hepatic Fibrosis, **Early Fibrosis** (\leq NASH CRN F2),
- ICD10-K74.02: Hepatic Fibrosis, **Advanced Fibrosis** (\geq NASH CRN F3),

Reference Biomarker: NASH-CRN Fibrosis Stages

N= 709

Ph-FCS Cut off= 5

Specificity: 86.62%

Sensitivity: 87.65%

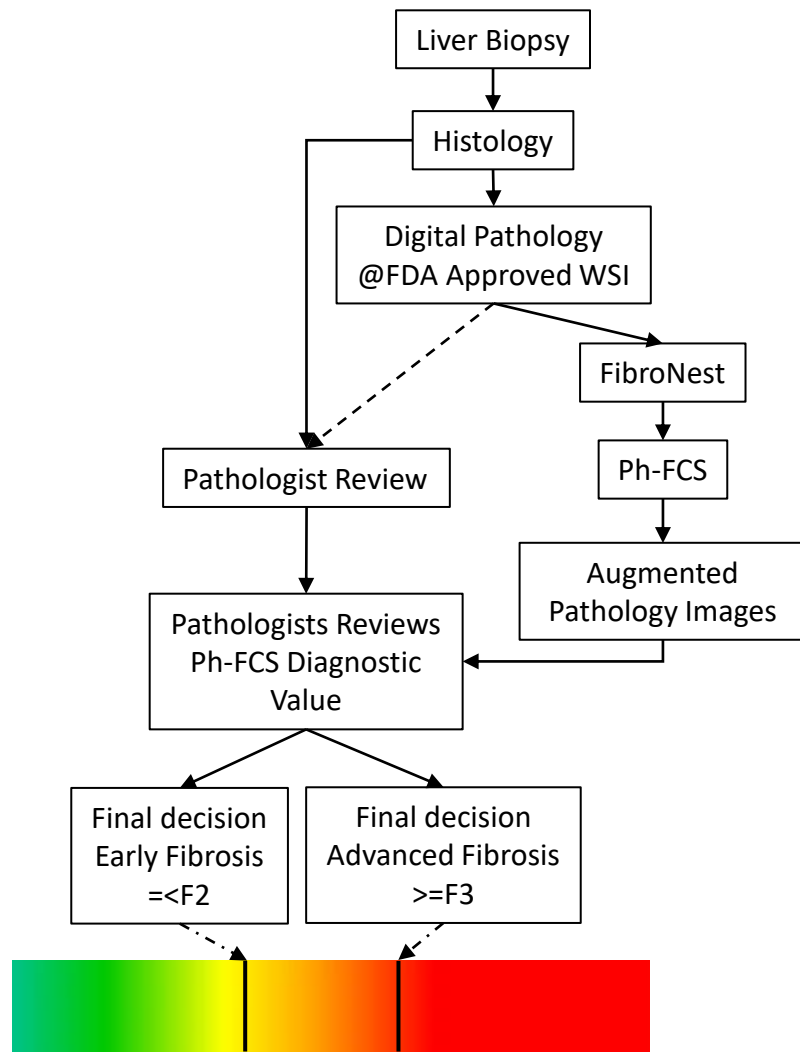
PPV: 98.19%

NPV: 45.98% *

* The performance or the Ph-FCS might be affected by the accuracy of the Reference Biomarker

Ph-FCS Fibrosis Biomarker

Ph- FCS Biomarker utility in pre-cirrhotic NASH Clinical Studies



In the context of pre-cirrhotic NASH clinical studies, the Ph-FCS Fibrosis biomarker will:

Improve the quality of the NASH Studies primary end points:

- Aid pathologists adjudicate NASH-CRN Fibrosis stages in the F2-F3 transition zone

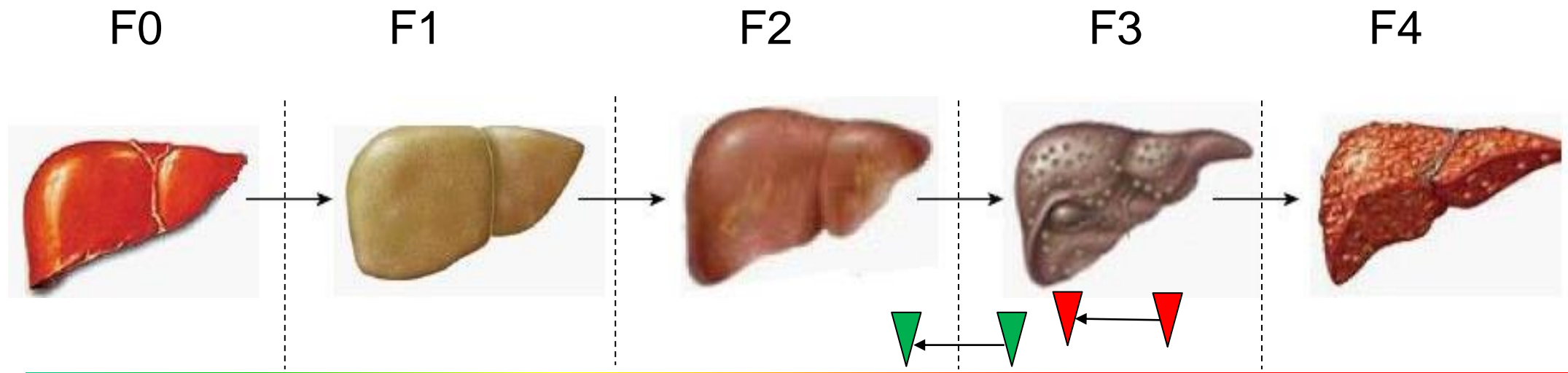
Generate an exploratory and continuous outcome for fibrosis severity to

- Describe base-line characteristics
- Quantify the effect of an intervention using mean-change from baseline data analysis paradigms

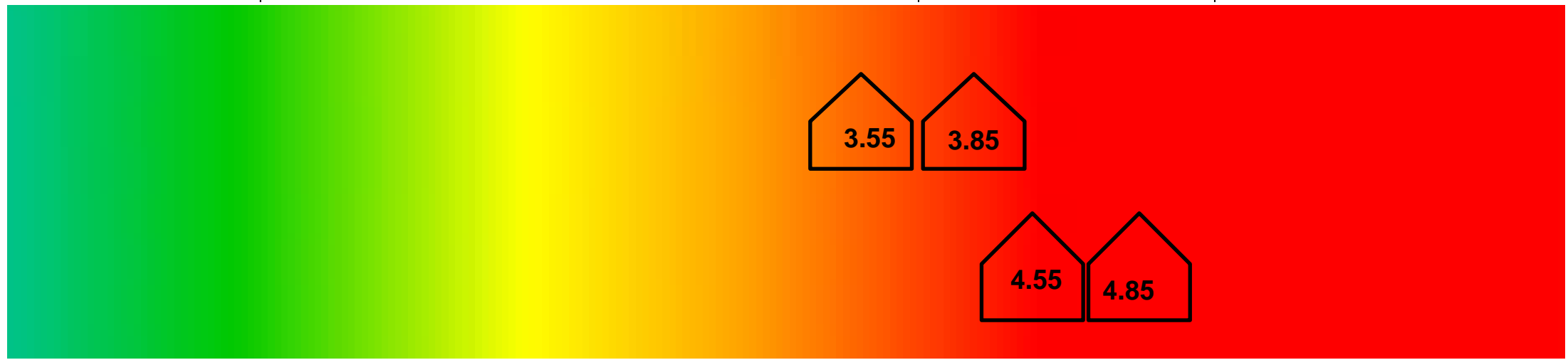
Clinical Applications of Ph-FCS

Continuous Phenotypic Score for Fibrosis Severity

Existing Paradigm



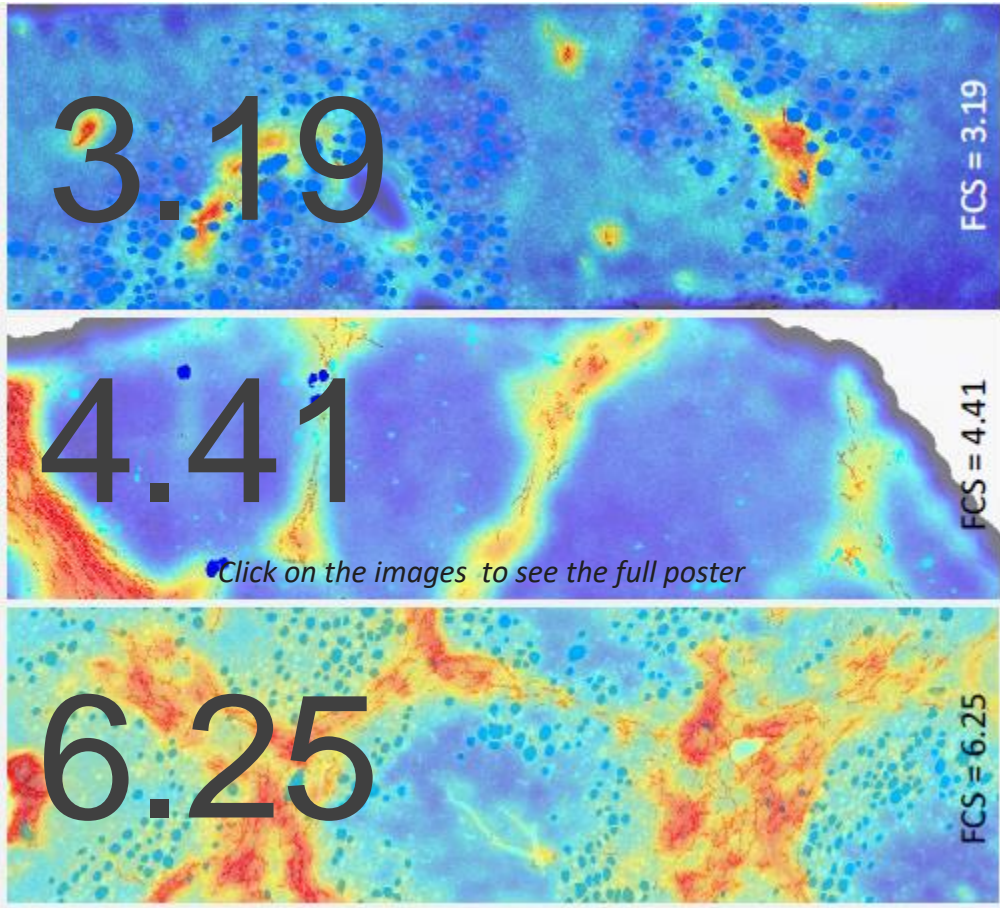
New Paradigm



Continuous Score measure the true effect of an intervention and change the Data Analysis Paradigm

Clinical Applications of Ph-FCS

Identification of responders in the context of Drug Development *(Aramchol, NCT02279524)*

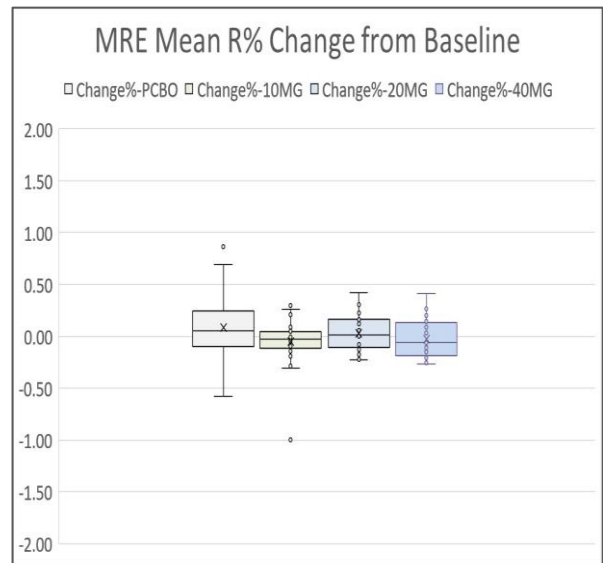
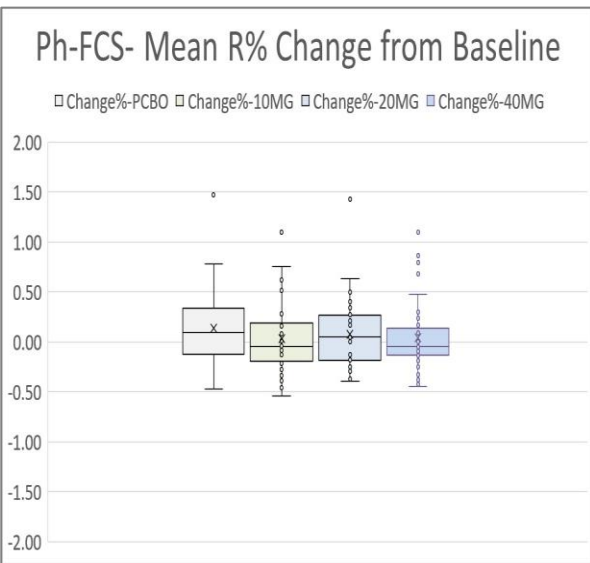
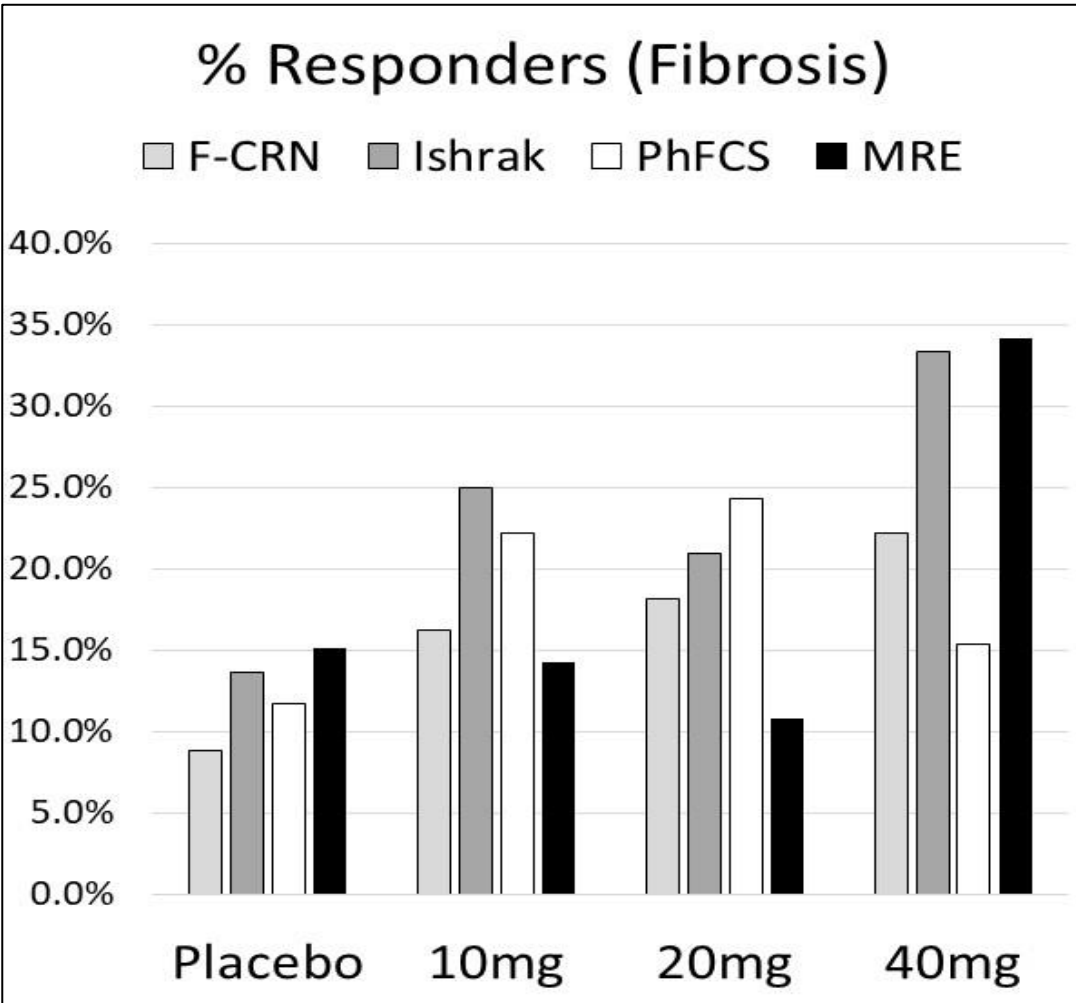


Biopsy methodology	Post-BL Biopsy at <W48 weeks		Post-BL Biopsy at ≥ W48	
	N	%	N	%
All	28	100%	23	100%
Fibrosis Improvement (1 point or more) based on NASH CRN	7	25%	9	39%
Fibrosis Improvement (Paired reading ranked assessment) based on comparing individual patients slides	12	43%	14	61%
Subject Fibrosis Response (AI reading) using Fibronest's Phenotypic FCS (A responder is defined by an absolute reduction of >0.3)	15	54%	23	100%
Subject Fibrosis Response (AI reading) using Fibronest's Phenotypic FCS (A responder is defined by a relative reduction of 25%)	6	21.4%	15	65.2%

FibroNest Fibrosis Continuous Biomarkers resolve paired-biopsy drug effect “inside” Categorical Scores

Clinical Applications of Ph-FCS

Identification of responders in the context of Drug Development



Pharmacodynamic Response | Ph-FCA Mean Change from Baseline | MRE Mean Change from Baseline : Ph-FCS benchmarks MRE response

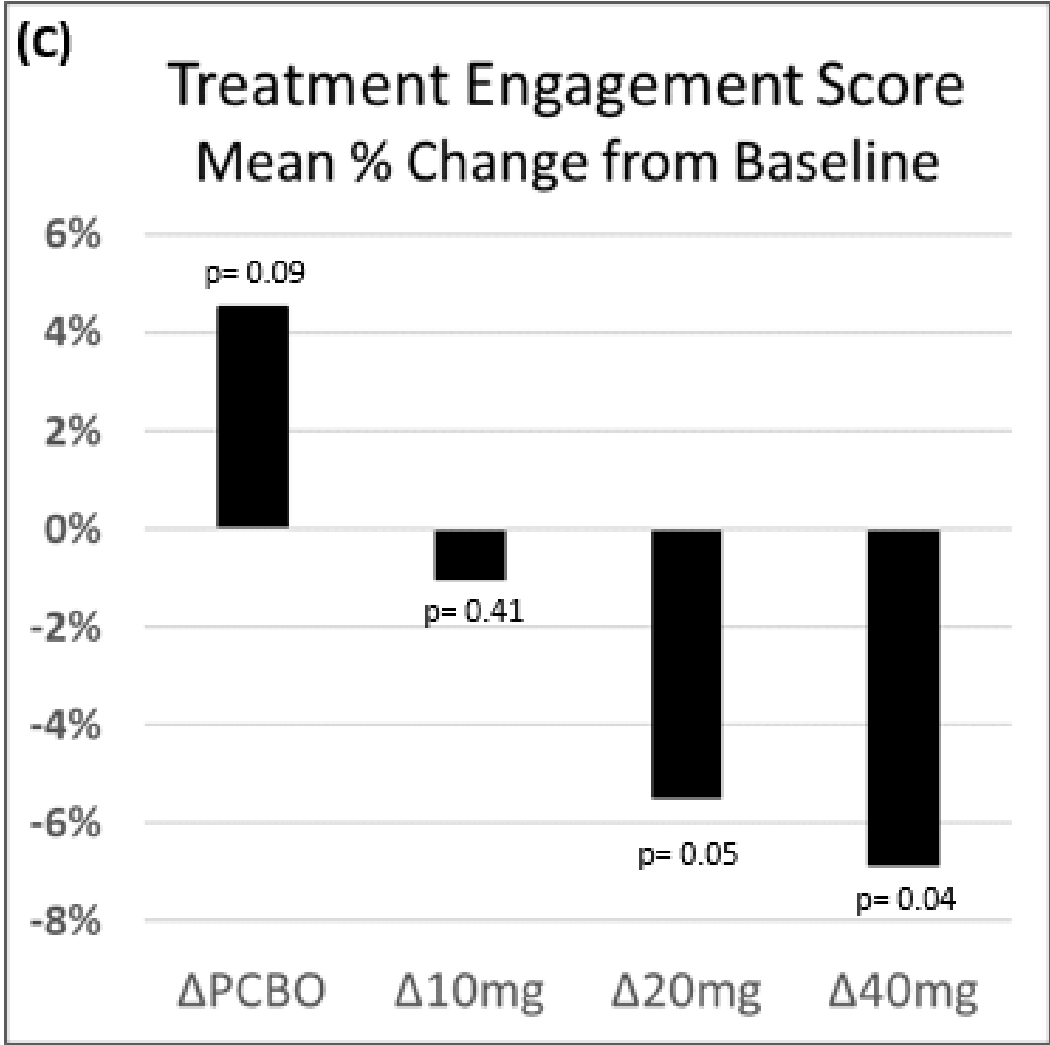
Ph-FCS detects the treatment effect of NASH drug candidates with a performance that benchmarks Imaging based measurements

Pharmacodynamic Response : Responders: NASH-CRN: 1 stage | Ph-FCS: 25% relative reduction | MRE: 15% relative reduction

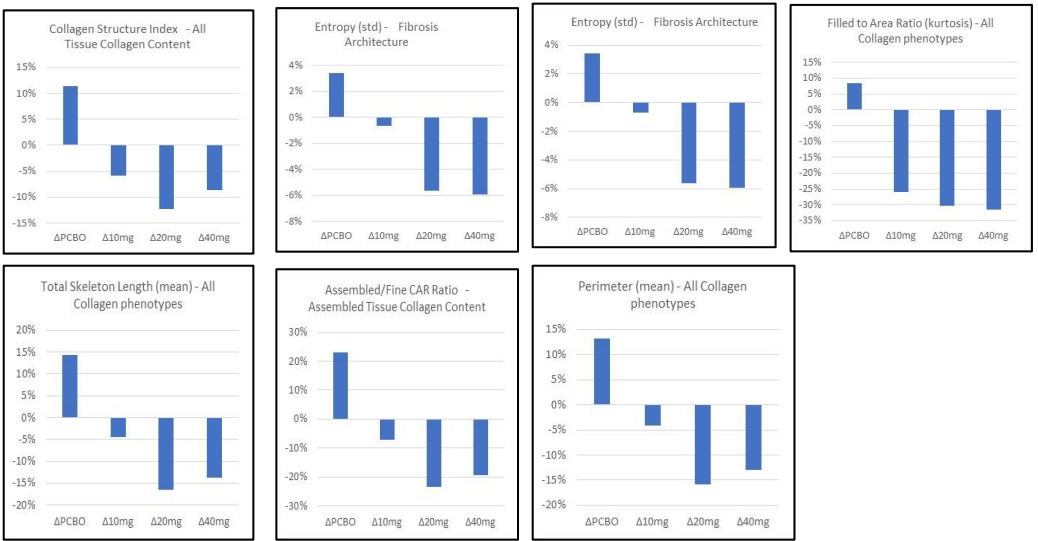
Novel Digital Pathology quantitative image analysis and AI method detects the treatment effect of NASH drug candidates with a performance that benchmarks Imaging based measurements. Li Chen (1), Elizabeth Brown (2), Anne Minnich (2), Vipul Baxi (2), Dimple Pandya (2), Edgar D. Charles (2), Zachary Goodman (3), Shuyan Du (2), Mathieu Petitjean (1), Arun J. Sanyal (4), (1) Pharmanest, Princeton, NJ, USA (2) Bristol Myers Squibb, Princeton, NJ, USA (3) Inova Health Systems, Falls Church, VA (4) Virginia Commonwealth University, Richmond, VA, USA ([poster link here](#))

Clinical Applications of Ph-FCS

Detection of Pharmacodynamic Response (*Pegbelfermin*, NCT03486899)



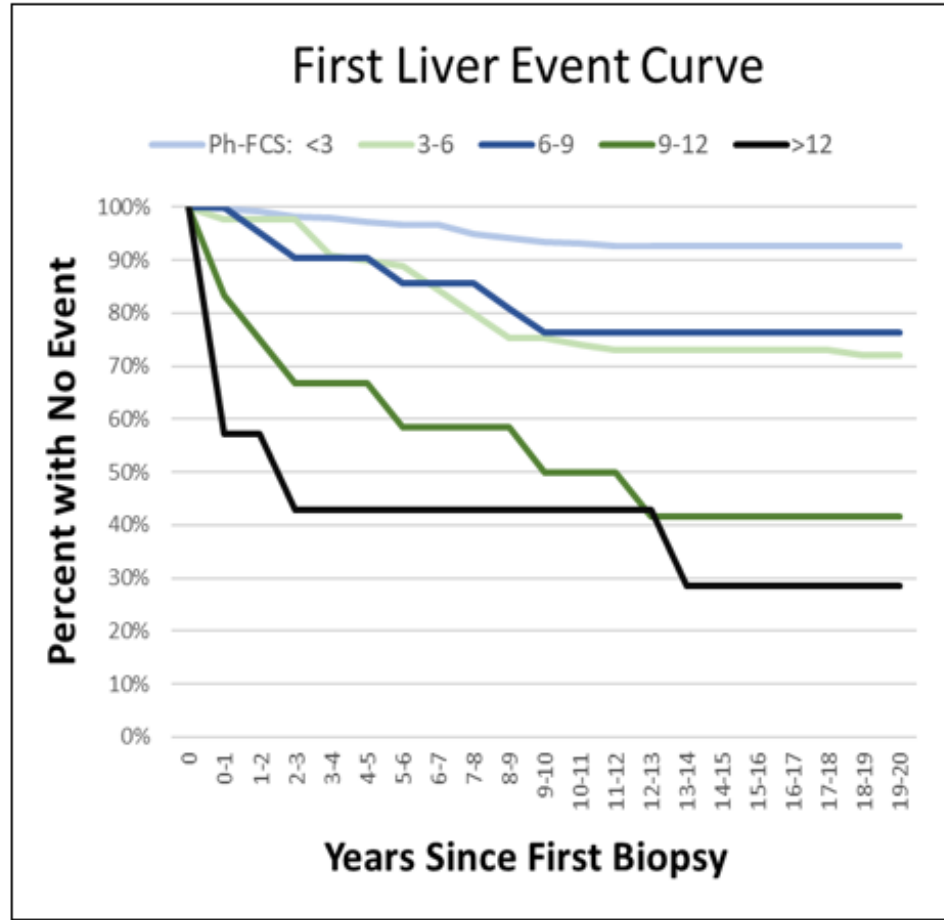
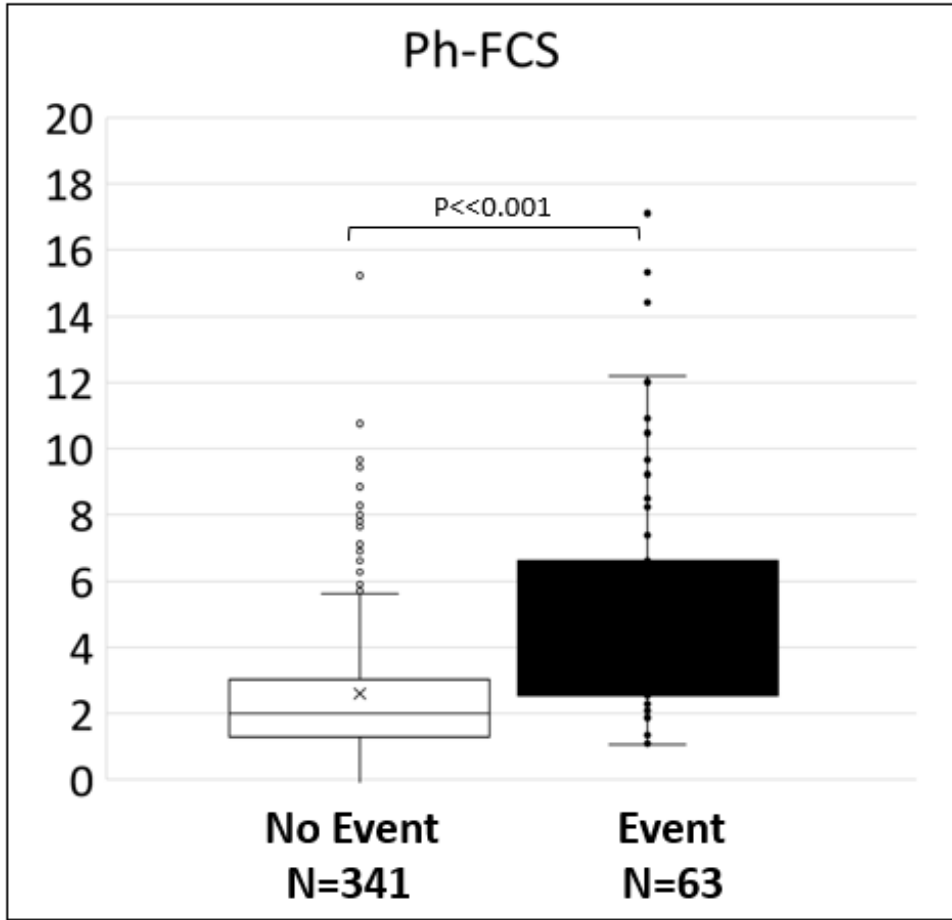
25 Phenotypic traits of Fibrosis are engaged during the 24 weeks intervention with Pegbelfermin



Novel Digital Pathology quantitative image analysis and AI method detects traits of fibrosis treatment response / pharmacodynamic Response

Clinical Applications of Ph-FCS

Prediction of clinical Liver related Events



Results: Mean age was 53.5 yrs, 56% were males, mean BMI 30.6 kg/m², 39% had diabetes and 62% arterial hypertension. The proportion of histological fibrosis stages were: 0/1/2/3/4, 53%/17%/8%/14%/8%, respectively. Median follow-up was 11.4 yrs (IQR 4.7). 52 pts (17%) had at least one LRE. Mean (median | sd) Ph-FCS was 5.19 (3.91 | 3.74) in pts with LRE vs 2.60(2.00|2.22) in pts without LRE (p<0.001). Using a cut-off value of 3, Ph-FCS had a sensitivity of 66.6% and specificity of 74.5% for the prediction of LRE. When the cut-off value was changed by +/-5% the sensitivity and specificity varied within a -4.8% to +2.8% range. The related Kaplan Myer curve describing the occurrence of the first LRE with time following the biopsy used to establish the Ph-FCS demonstrates that the LRE risk can be stratified according to the Ph-FCS value.

Ph-FCS (N=404)			Sensitivity Analysis	
Cut off	Sensitivity	Specificity	Sensitivity	Specificity
2.85	68.25%	71.55%	2.38%	-3.94%
3.00	66.67%	74.49%		
3.15	63.49%	76.54%	-4.8%	2.8%

Ph-FCS predicts Liver Events (N=404, 18years of follow up, AUROC=0.76).

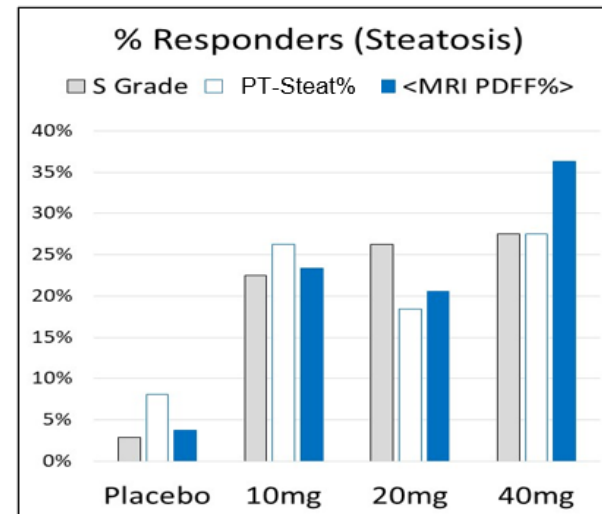
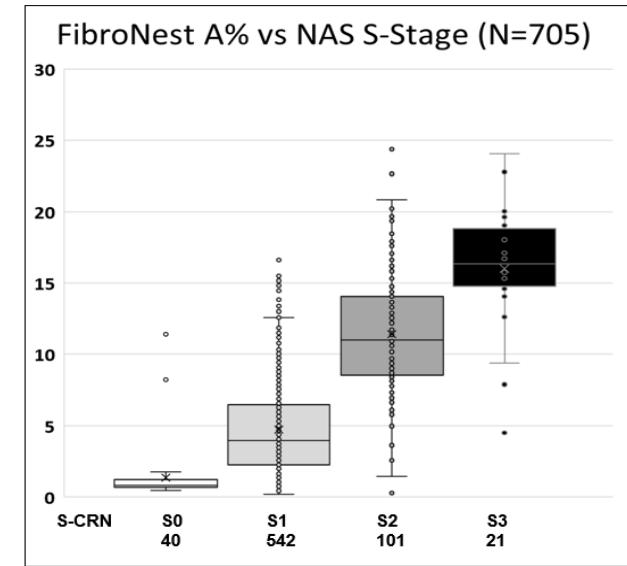
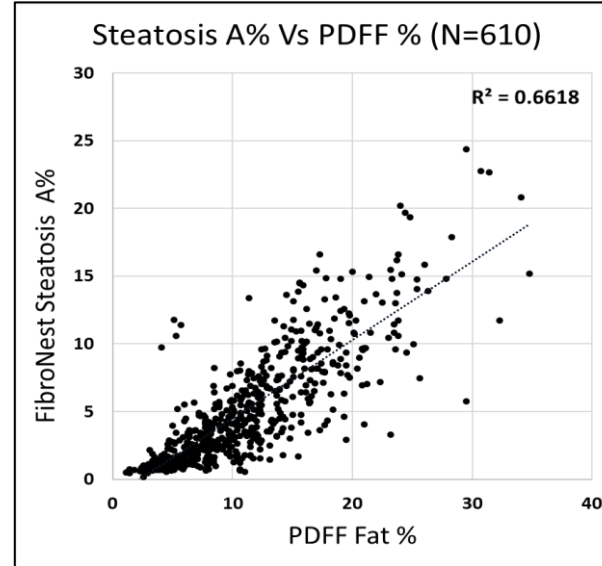
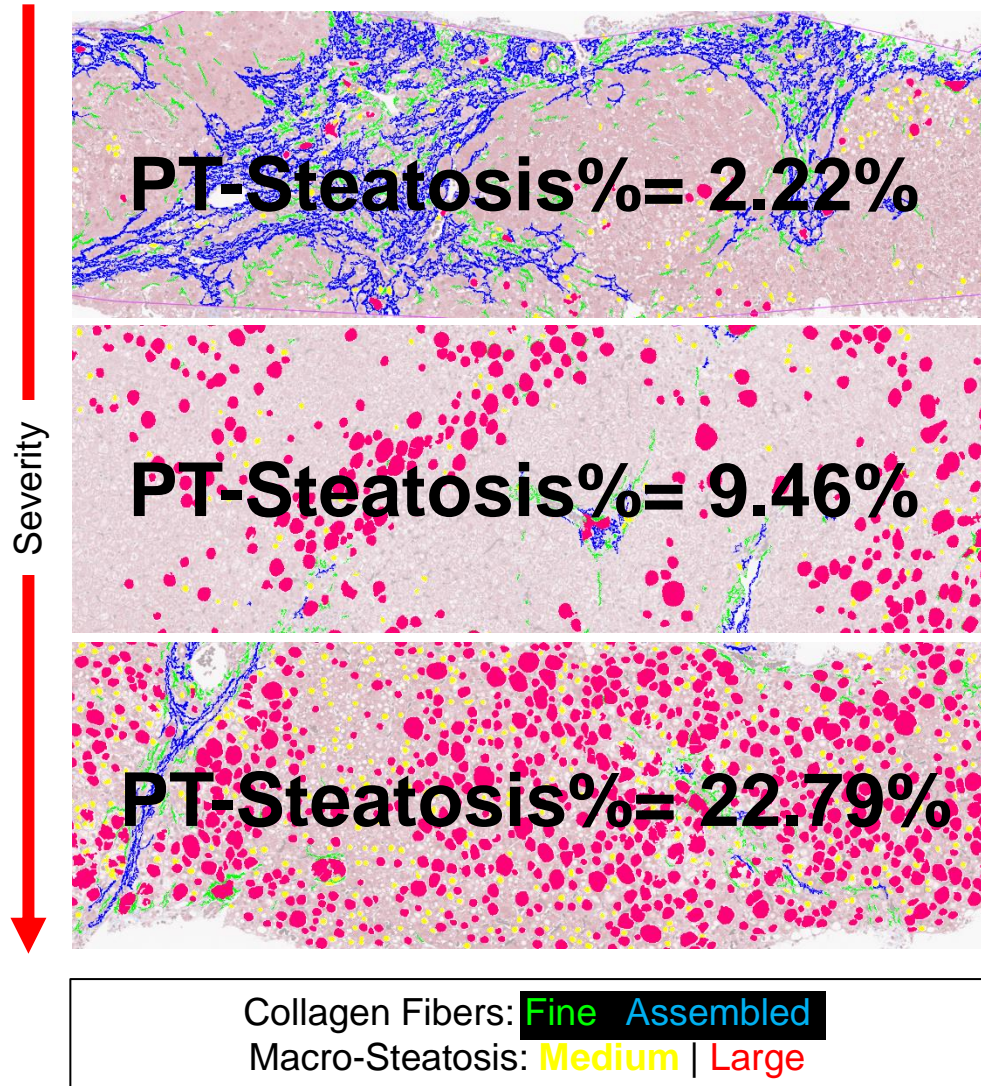
Novel artificial intelligence-assisted digital pathology quantitative image analysis predicts the occurrence of liver-related clinical events in the multicentric, European, Hepatic Outcomes and Survival Liver Registry (HOTSURFR)

study. Li Chen¹, Louis Petitjean¹, Javier Ampuero², Jerome Boursier³, Stergios Kechagias⁴, Salvatore Petta⁵, Hannes Hagström⁶, Jörn Schattenberg⁷, Frederic Charlotte⁸, Leila Kara⁹, Pierre Bedossa¹⁰, Mathieu Petitjean¹, Vlad Ratziu¹¹

Clinical Validation and Applications of PT-Steatosis%

Identification of Steatotic effect in the context of Fibrotic Tissues

Definition: % of MacroSteatosis on Non-Fibrotic Tissue

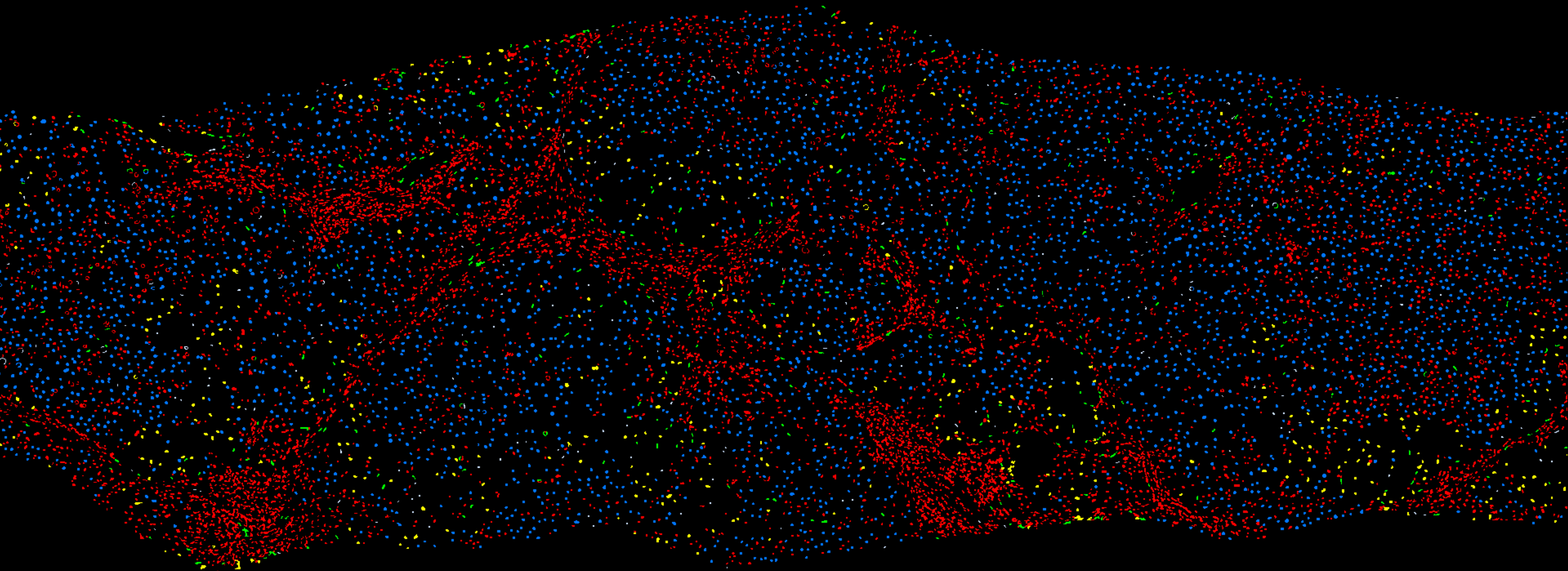


Correspondence with NASH CRN Scores (N=705, FALCON 1, 2 and LIFT), and with MRI PDFF . N=610 (Falcon 1-2)

Pharmacodynamic Response : Responders identified:
 NASH-CRN: 1 stage | A% : 30% relative reduction | PDFF: 30% relative reduction (FALCON 1 Phase 2 Study)

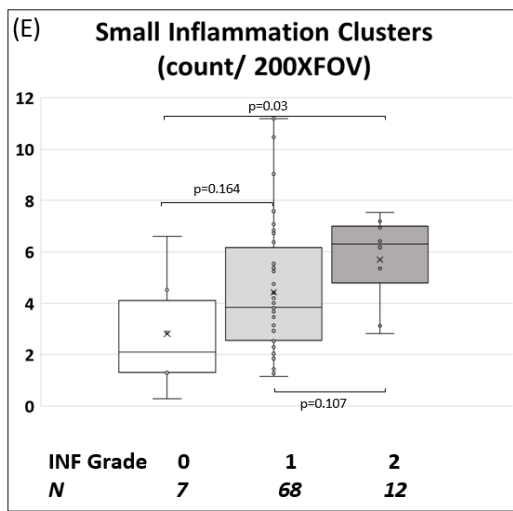
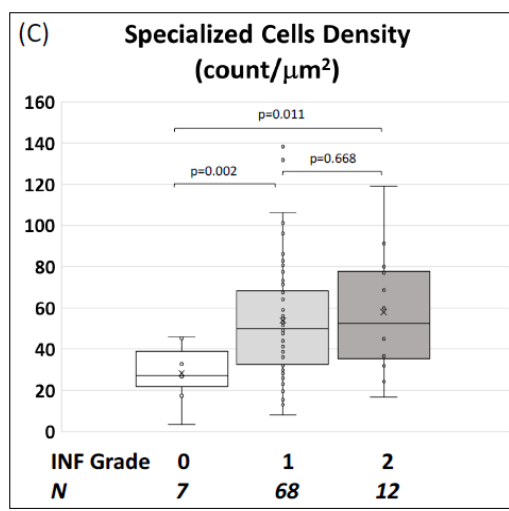
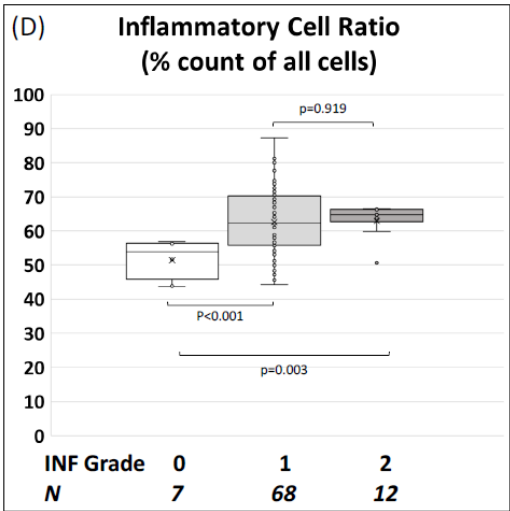
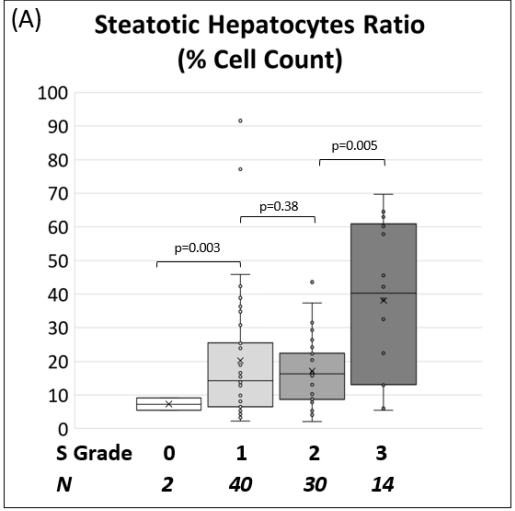
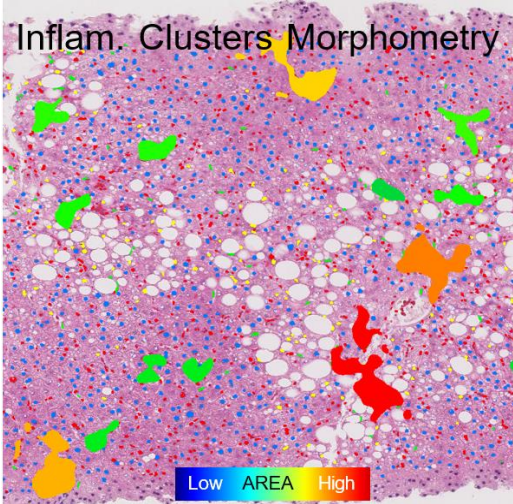
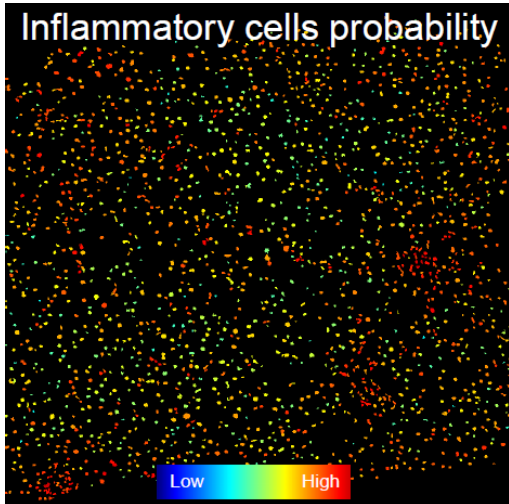
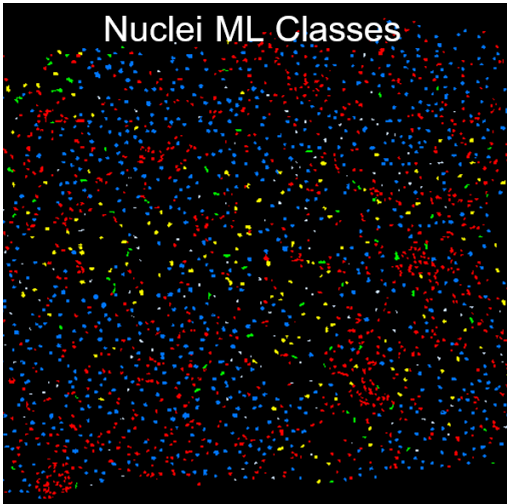
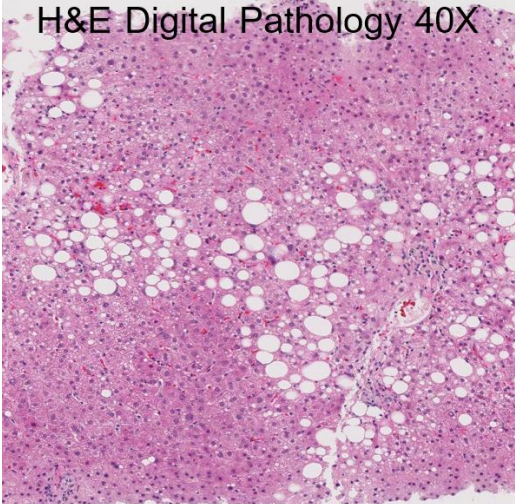
Pharmacodynamic Response : A% Mean Change from Baseline | PDFF Mean Change from Baseline: A% benchmarks PDFF response. (FALCON 1 Phase 2 Study)

Single Cell Tissue Pannels & Inflammation



Normal Hepatocytes
Steatosis Hepatocytes
Inflammatory Cells
Specialized Cells

Quantitative Image Analysis and AI classification: A quantitative approach to Tissue Injury

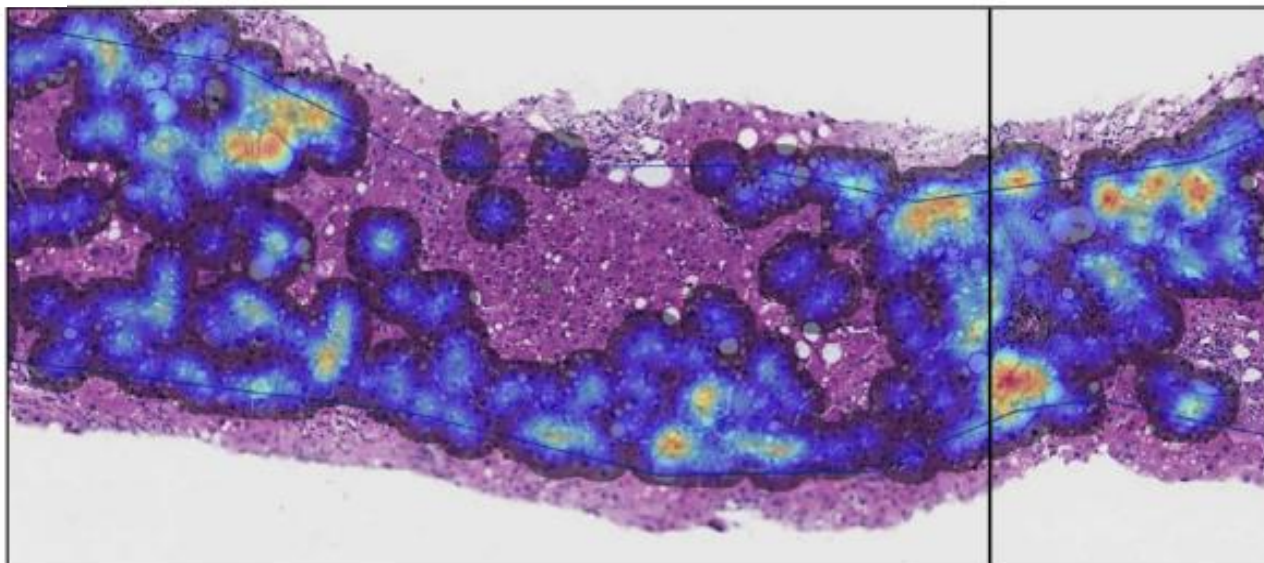


“NASH-CRN” Steatosis

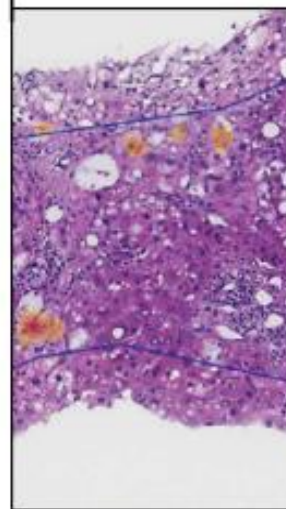
“NASH-CRN” Inflammation

Lobular Ballooning Quantification (Machine Learning from Pathologist Annotations)

0% - 100% probability map



Probability of hepatocyte ballooning

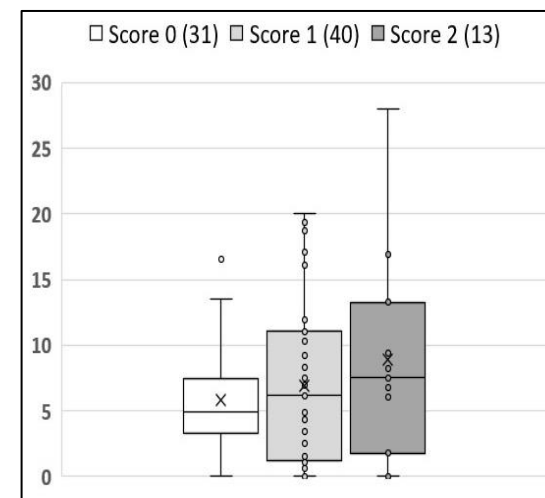


75% - 100% probability map

FibroNest Analytical Hypothesis:

- Ballooned Cells are the expression of a “*Balloonification*” process
- Pathologists annotated with a “confidence Level”
- AI Model generate a confidence Level and only >75% is accepted
- Cluster are phenotypes of severity, according to some pathologists, but not all

Results



Limited Performance

Limitations

Research Article
NAFLD and Alcohol-Related Liver Diseases
JOURNAL OF HEPATOLOGY
**Complexity of ballooned hepatocyte feature recognition:
Defining a training atlas for artificial intelligence-based
imaging in NAFLD**

Upcoming Data Generation Plan

To support Biomarker Clinical Validation and advance of the field of Fibrosis



- **N=2000** NASH Liver WSI Biopsies
- Retrospective Analyses by Q1 2024

- Validation vs Pathologists

- NITS development

- Pr. Quentin Asntee
- LITMUS Investigators
- LITMUS Industry Partners

Europe



- **Phase2 Program**
- **N=2000** NASH Liver WSI Biopsies
- Timing : per NIMBLE

- Validation vs Pathologists
- Possible Liver Outcomes

- NITS development

- Pr. Arun Sanyal
- NIMBLE Investigators
- NIMBLE Industry Partners

USA

Recap / Summary

- ❑ **Same Slide(s) as Pathologist**
- ❑ **High Resolution**
- ❑ **High content | Single Fiber | Single Cell Image Analysis**
- ❑ **AI > large quantitative & relevant data-lakes**

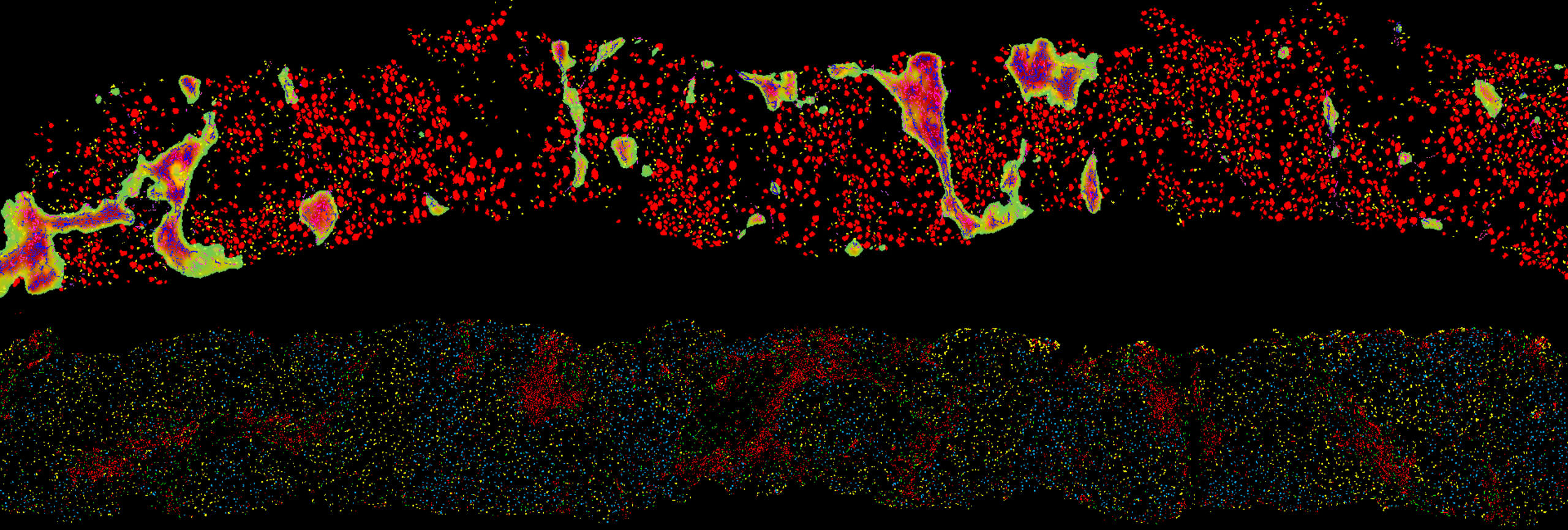
- ❑ **Robust vs Pre-Analytical condition**
- ❑ **Compatible with FDA approved WSI scanners**

- **Improve adjudication**
- **Describe baseline characteristics**
- **Detect responders “inside” categorical stages**
- **Detect treatment responder / pharmacodynamic response**
- **Predict Liver Related outcomes (POC, N=400)**

- ❑ **Currently engaged with FDA / CDER - BQP**
- ❑ **3 Phase 3, 7 Phase 2 NASH, 3 Phase 2 in other conditions, 60+ preclinical**

Thank You !

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