



# 3<sup>rd</sup> Paris NASH Symposium

French-US Meetings

July 6 & 7, 2017

Institut Pasteur - Paris

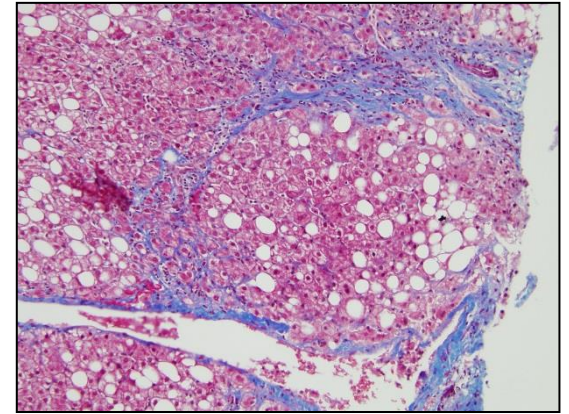
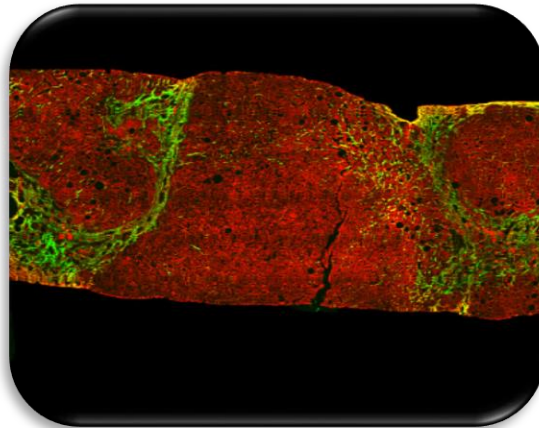
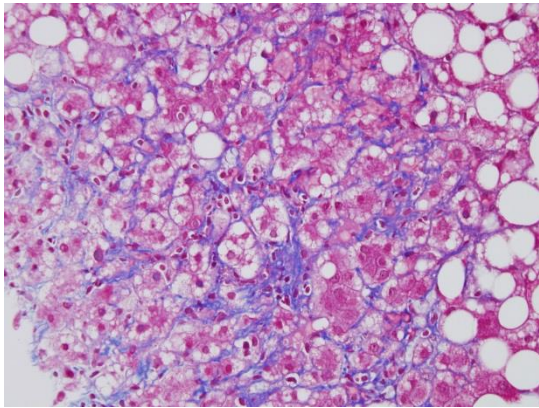
Organized by  
Arun Sanyal & Lawrence Serfaty

Virginia Commonwealth University School of Medicine, Richmond, Virginia, US  
Hôpital Saint-Antoine, APHP, Inserm, Université Pierre & Marie Curie, Paris, France

With the partnership of



# Dual photon microscopy and its potential applications in NASH



Arun J. Sanyal MBBS, MD

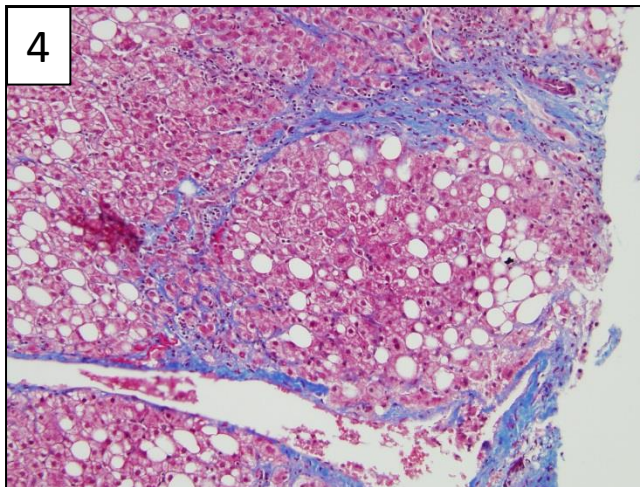
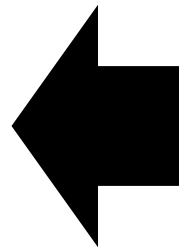
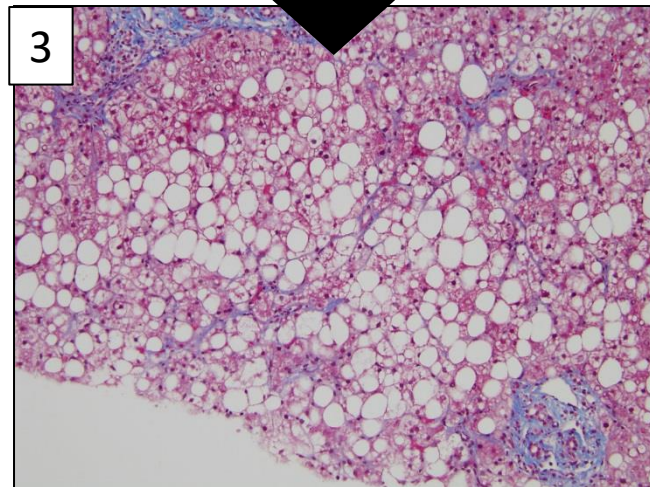
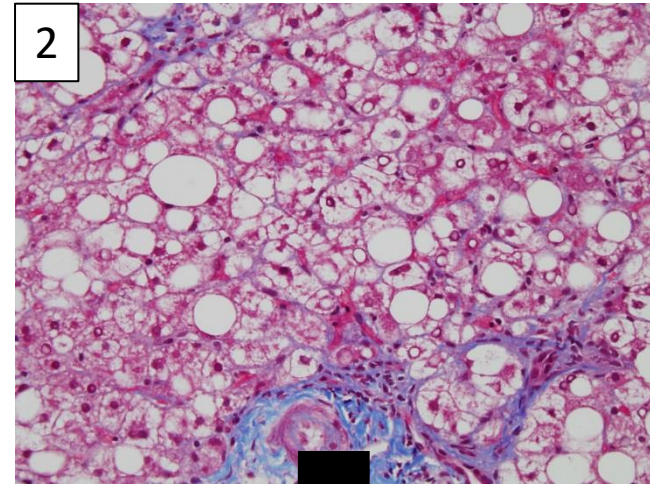
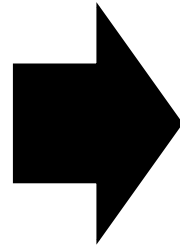
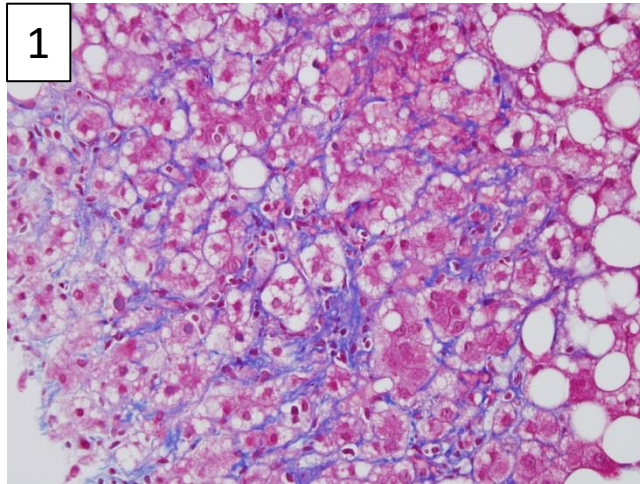
Professor of Medicine, Physiology and Molecular Pathology

Virginia Commonwealth University School of Medicine

# Conflicts of Interest

- President, Sanyal Biotechnologies
- **Stock options:** Genfit, Akarna, Tiziana, Indalo, Durect, Exhalenz, Hemoshear
- **Advisor with compensation:** Lilly, Pfizer, Novartis, Ardelyx, Salix, Hemoshear
- **Advisor without compensation:** Galectin, Intercept, Merck, Bristol Myers, Immuron, Gilead, Chemomab, Affimmune, Protalix, Nitto Denko, Novo Nordisk, Cirius, Boehringer Ingelhiem
- **Grants to institution:** Gilead, Tobira, Allergan, Merck, Bristol Myers, Astra Zeneca, Immuron, Intercept, Novo Nordisk, Shire, Boehringer Ingelhiem, Cirius

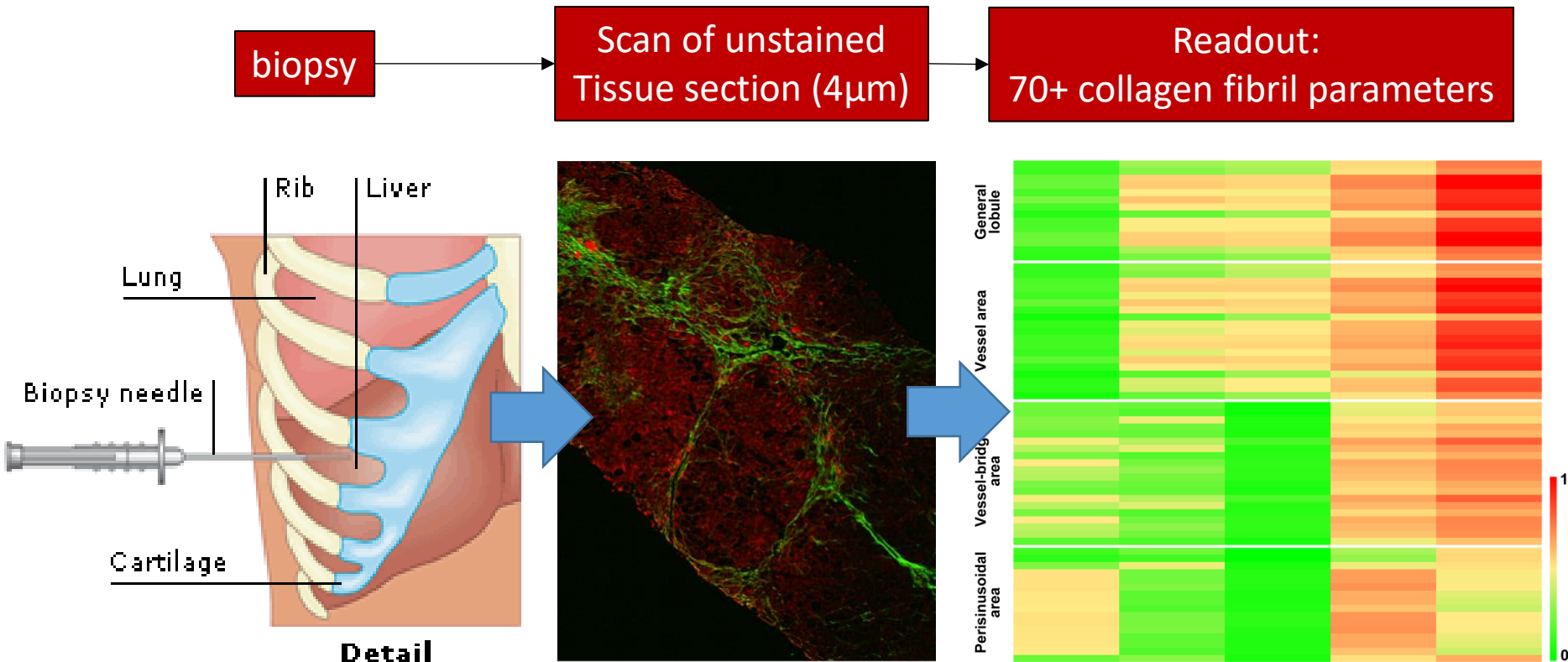
# Histological Staging of NASH



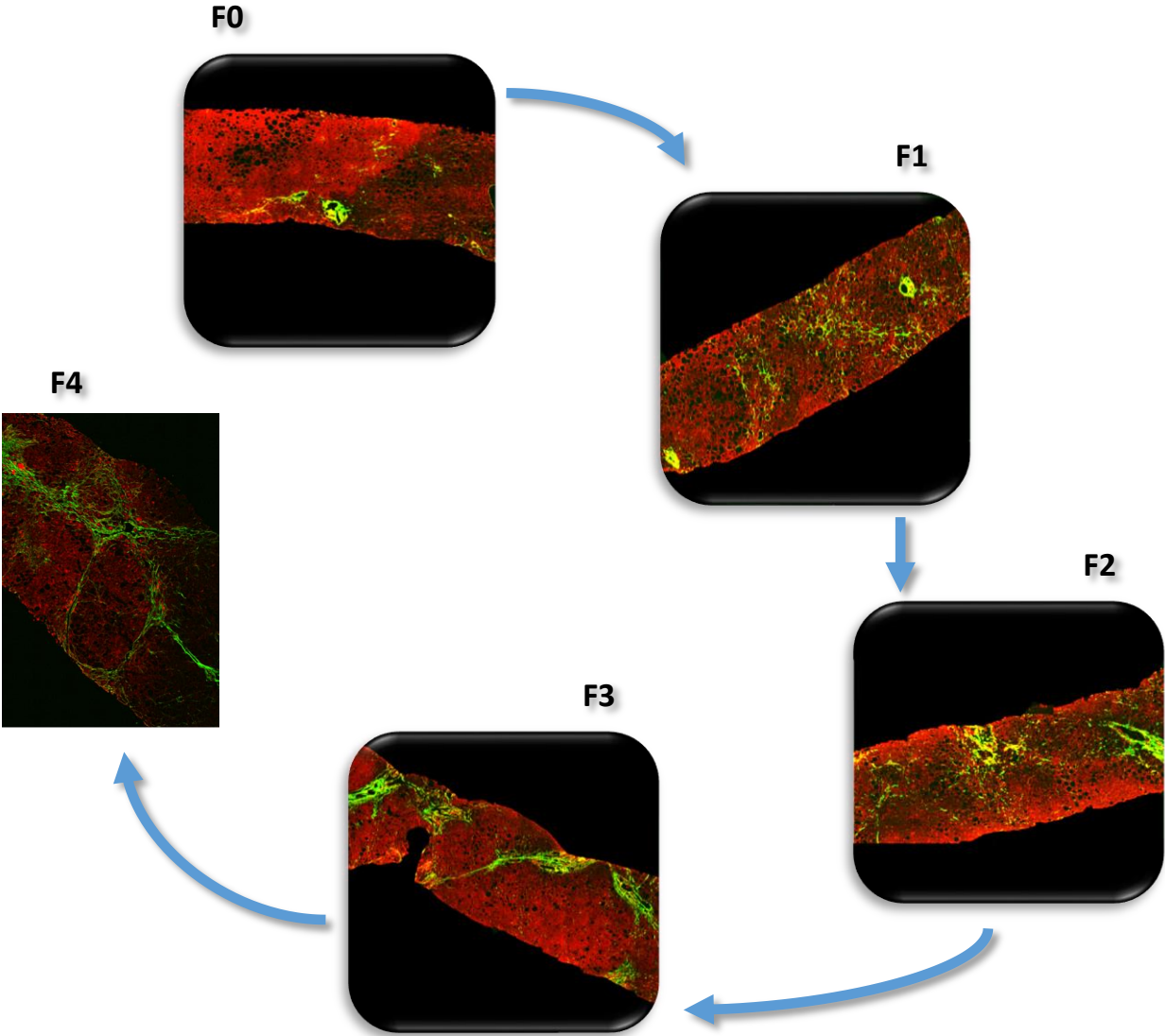
# Limitations of traditional methods of histological staging of fibrosis

- Susceptibility to sampling variability
- Intra- and Inter-observer variability
- Ordinal classification of a continuous process
- Limited dynamic scale

# Workflow for Dual Photon Microscopy of the liver

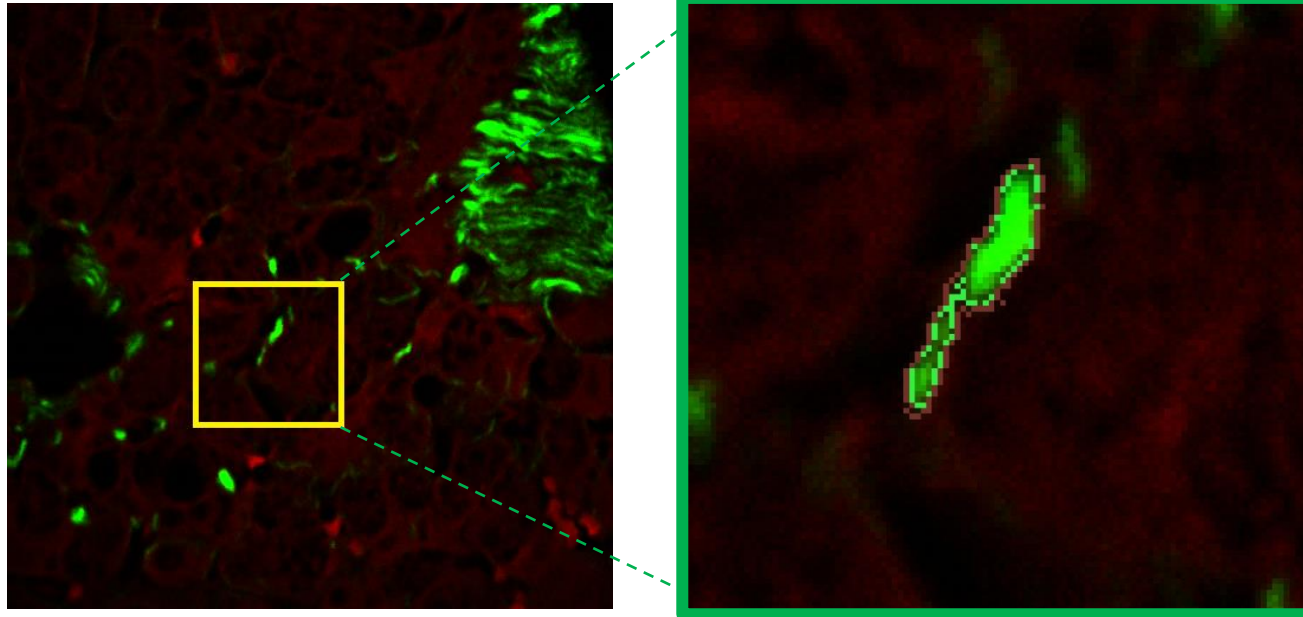


# Dual photon scan of the liver in NASH



# Dual photon microscopy permits measurement of collagen content and characteristics

## Collagen Quantification – Collagen Strings Morphometrics



- a. Area
- b. Perimeter
- c. Compactness -
- d. Length
- e. Breadth
- f. Convex Hull Perimeter
- g. Convex Hull Area

- h. Elongation
- i. Roughness
- j. Major Axis Length
- k. Minor Axis Length
- l. Orientation
- m. Axis Ratio
- n. Tortuosity



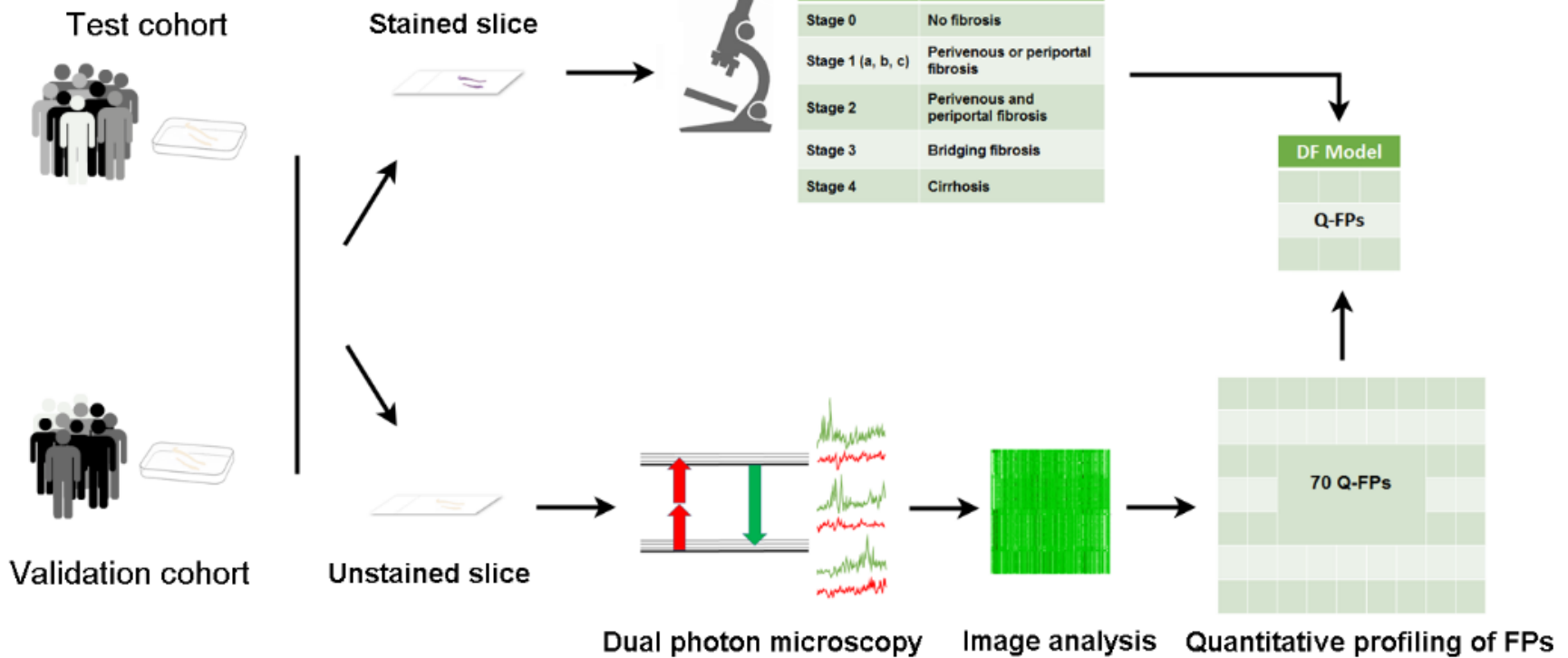
# Utility of histological methods of assessment

	Semiquantitative staging	Collagen proportionate area	Quantitative profiling of FP
Presence	Yes	Yes	Yes
Location	Yes	No	Yes
Extent	Descriptive ordinals	Quantitative value	Quantitative parameters
Dynamic scale	Limited	Continuous	?
Comparability (Q, SE, SP)	Standard	N/A	?
Applicability (Regulatory)	Routine practice, clinical study	Experimental, clinical study	Experimental, clinical study
Output	Descriptive estimation of fibrosis pattern and dynamics	Quantitative calculation of fibrosis dynamics	Quantitative calculation of fibrosis pattern and dynamics

# Establishment and Cross-sectional Validation of a New Tool for NAFLD

## NASH CRN fibrosis staging

Fibrosis stage	Fibrosis pattern
Stage 0	No fibrosis
Stage 1 (a, b, c)	Perivenous or periportal fibrosis
Stage 2	Perivenous and periportal fibrosis
Stage 3	Bridging fibrosis
Stage 4	Cirrhosis



# Basic characteristics



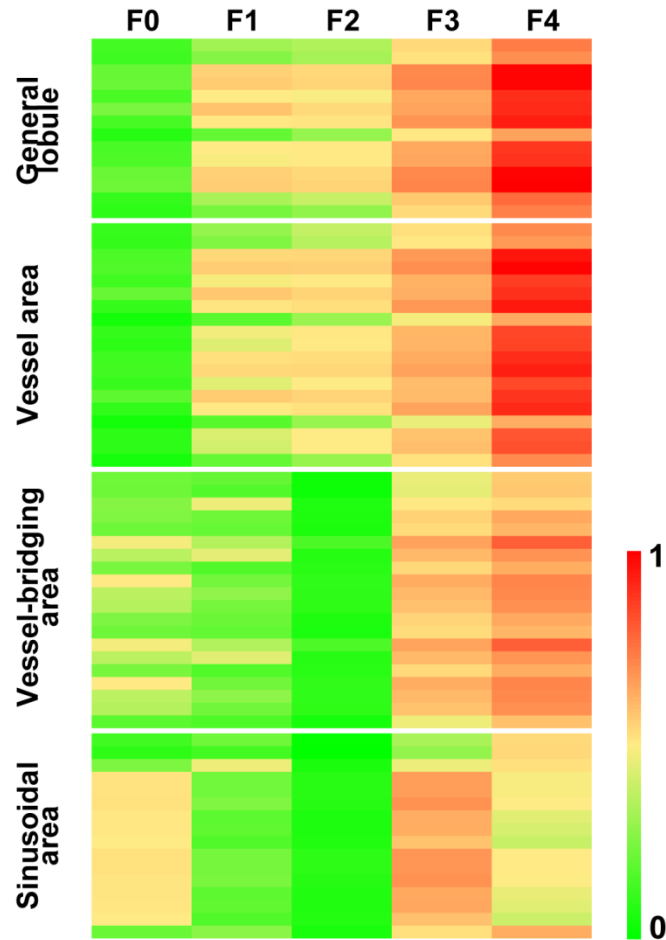
Variable	Test cohort (n=50)	Validation cohort (n=42)
	n (%) or median (range)	
Age (year)	56 (34-75)	57 (36-80)
Gender (male)	10 (20)	7 (17)
BMI (kg/m <sup>2</sup> )	33.8 (22.7-43.0)	34.4 (22.2-45.1)
BMI >30 kg/m <sup>2</sup>	40 (80)	34 (81)
Diabetes	21 (42)	20 (48)
Hyperlipidemia	32 (64)	30 (71)
ALT (U/L)	54 (13-255)	84 (24-248)
AST/ALT ratio	0.9 (0.5-4.3)	1.0 (0.4-3.7)
Biopsy length (mm)	16.5 (8.0-72.0)	15.5 (6.0-52.0)
<b>Steatosis</b>		
1	25 (50)	17 (40)
2	13 (26)	13 (31)
3	12 (24)	12 (29)
<b>Inflammation</b>		
0	2 (4)	2 (5)
1	26 (52)	32 (76)
2	22 (44)	8 (19)
3	0 (0)	0 (0)
<b>Ballooning</b>		
0	15 (30)	7 (17)
1	26 (52)	23 (55)
2	9 (18)	12 (29)



## Distribution of fibrosis stage

<b>Variable</b>	<b>Test cohort (n=50)</b>	<b>Validation cohort (n=42)</b>
	N (%)	
<b>Fibrosis stage</b>		
<b>0</b>	<b>9 (18)</b>	<b>5 (12)</b>
<b>1</b>	<b>12 (24)</b>	<b>13 (31)</b>
<b>2</b>	<b>12 (24)</b>	<b>10 (24)</b>
<b>3</b>	<b>7 (14)</b>	<b>12 (29)</b>
<b>4</b>	<b>10 (20)</b>	<b>2 (5)</b>

# Quantitative fibrosis parameters (qFP) change with increasing fibrosis stage

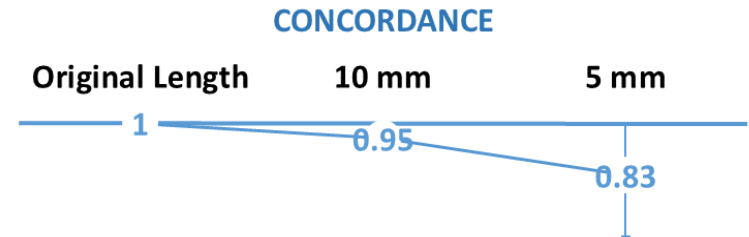


A total of 70 q-FPs:

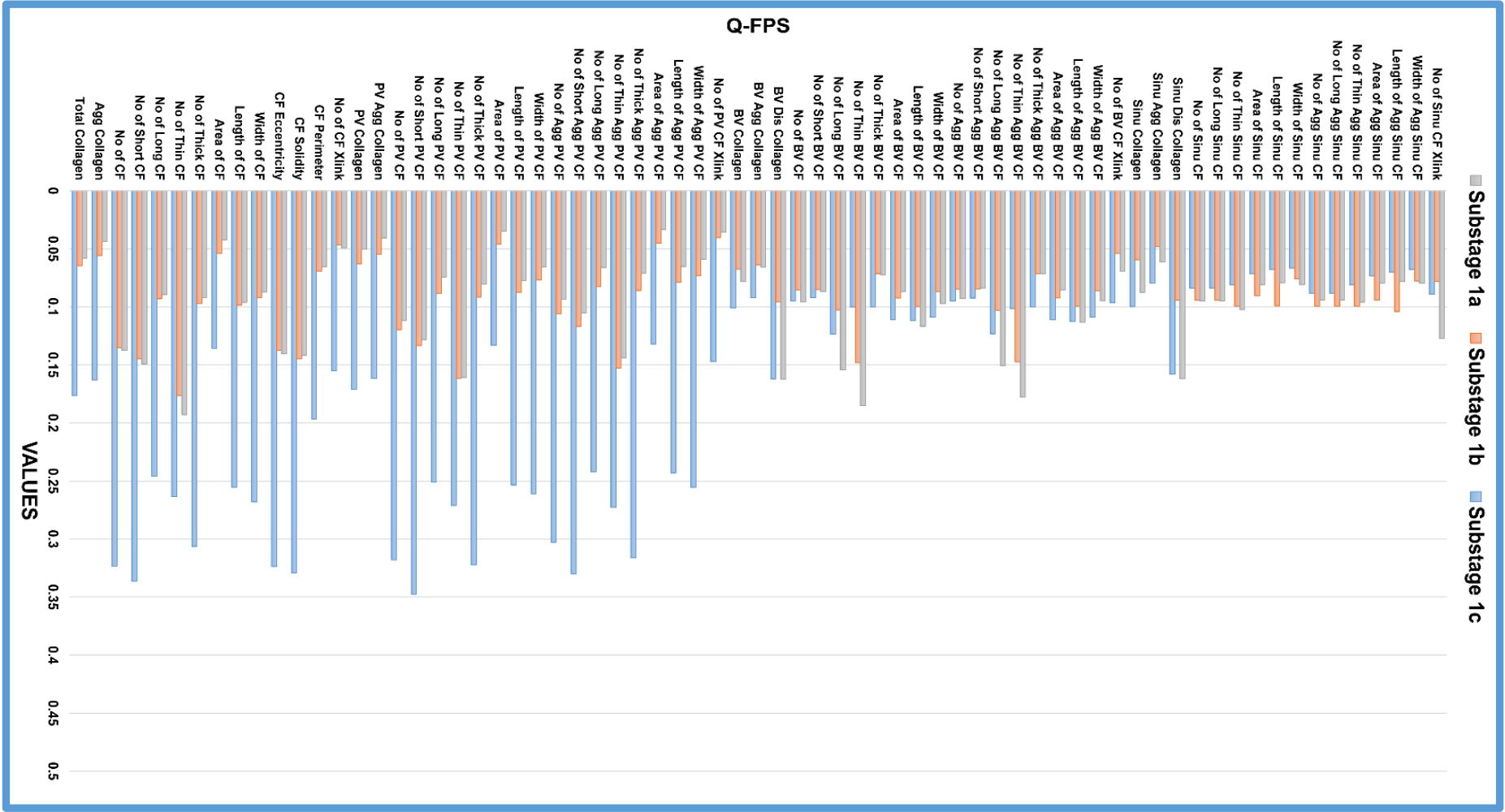
inter-observer agreement =  $0.97 \pm 0.03$

intra-observer agreement =  $0.98 \pm 0.03$

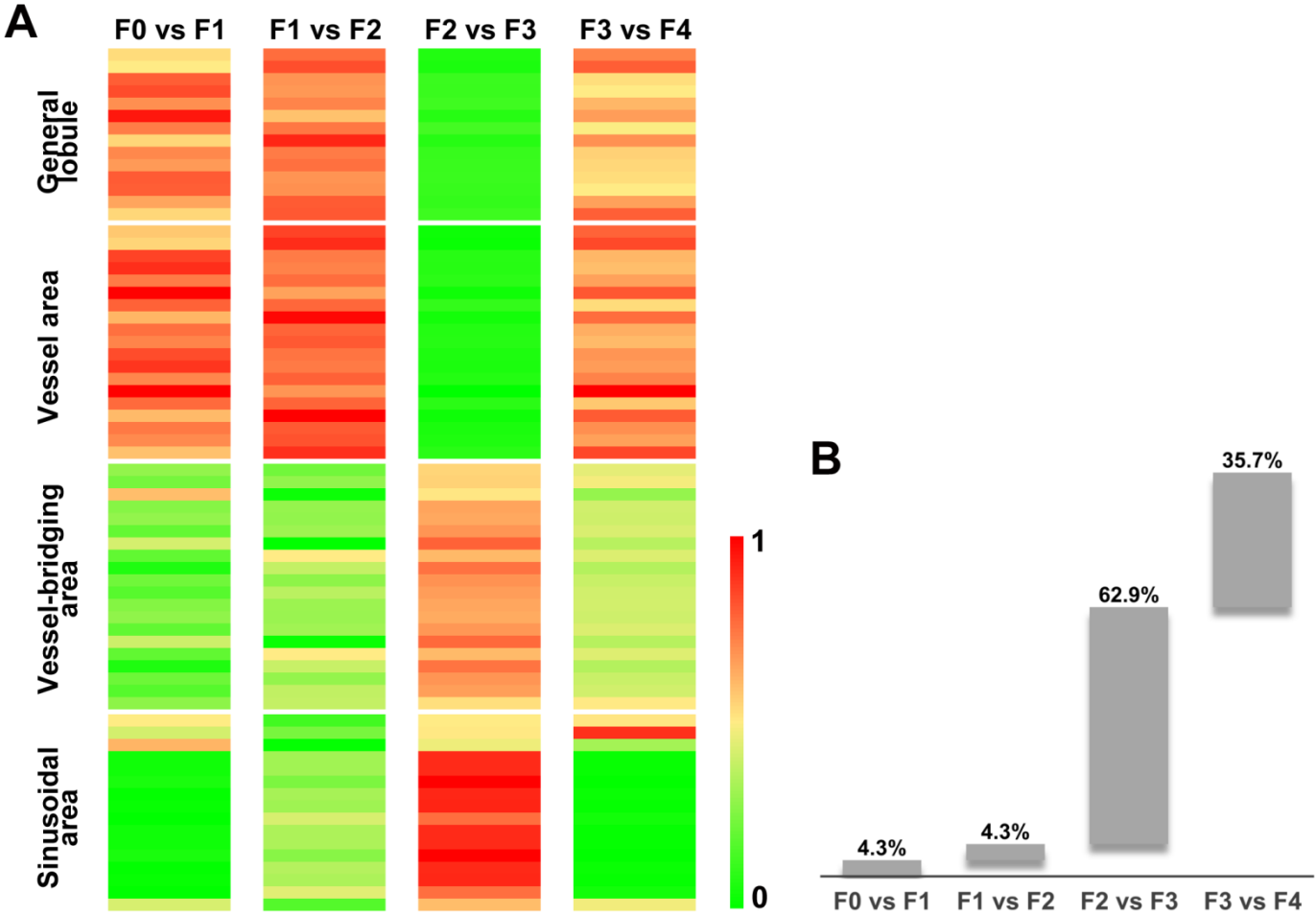
Sampling size variance:



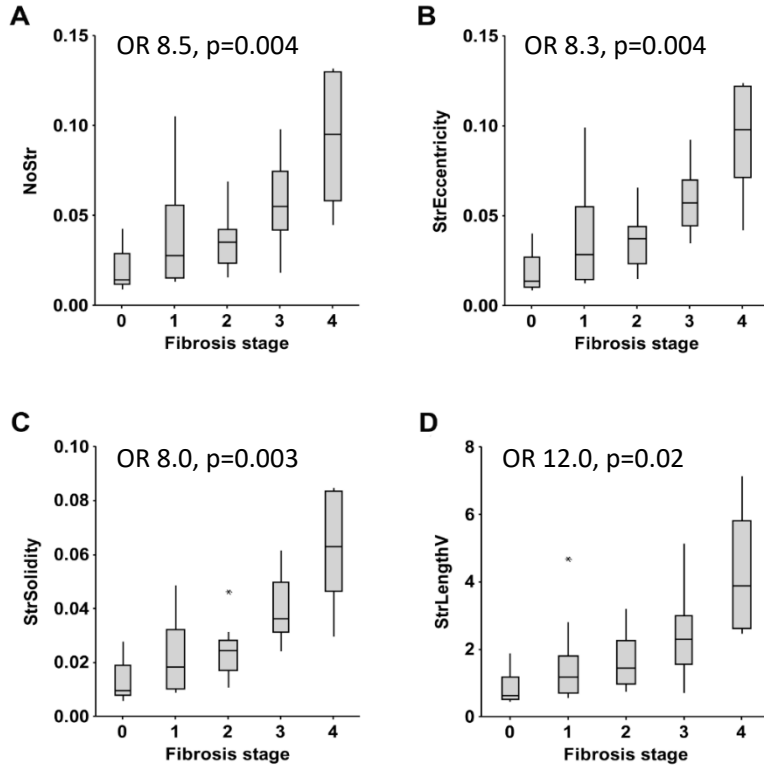
# qFP measures differences in collagen fibrillar properties even in stage 1 disease



# Visualization of changes in qFP with increasing fibrosis stage



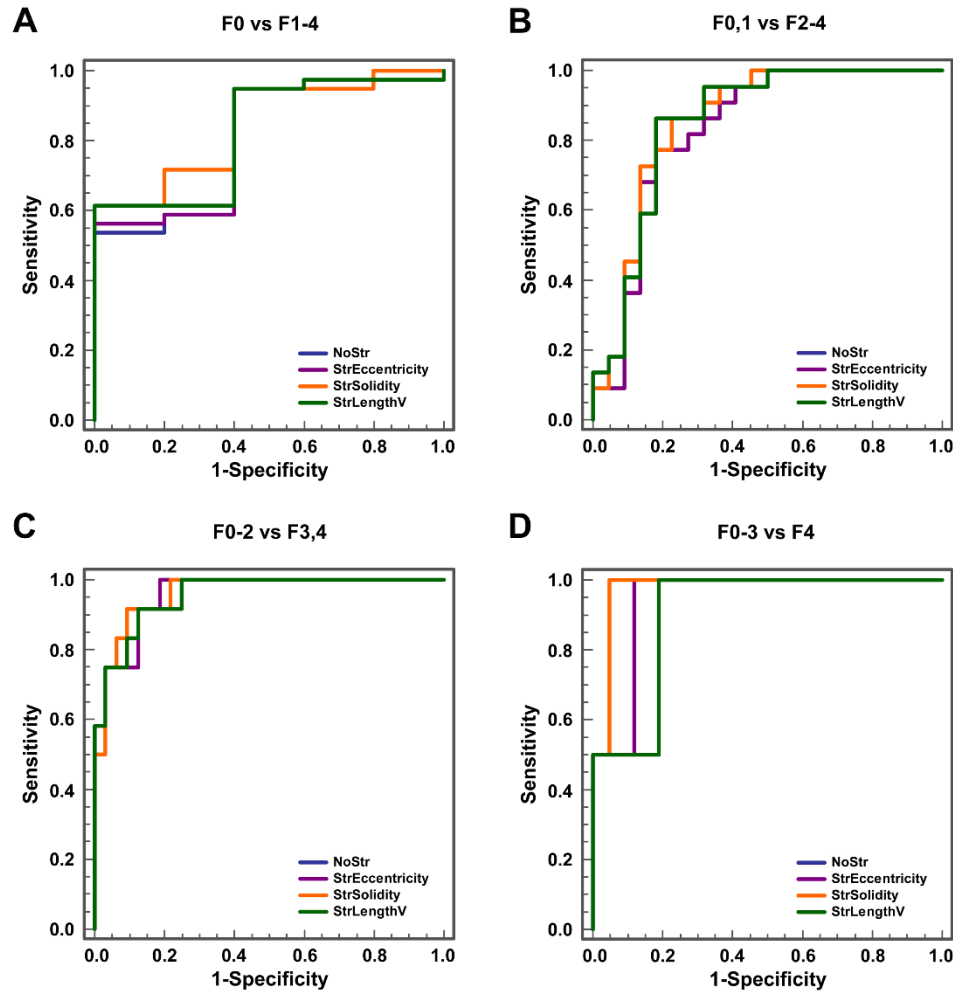
# Q-FPs with excellent performance for independently reflecting progressing NASH fibrosis



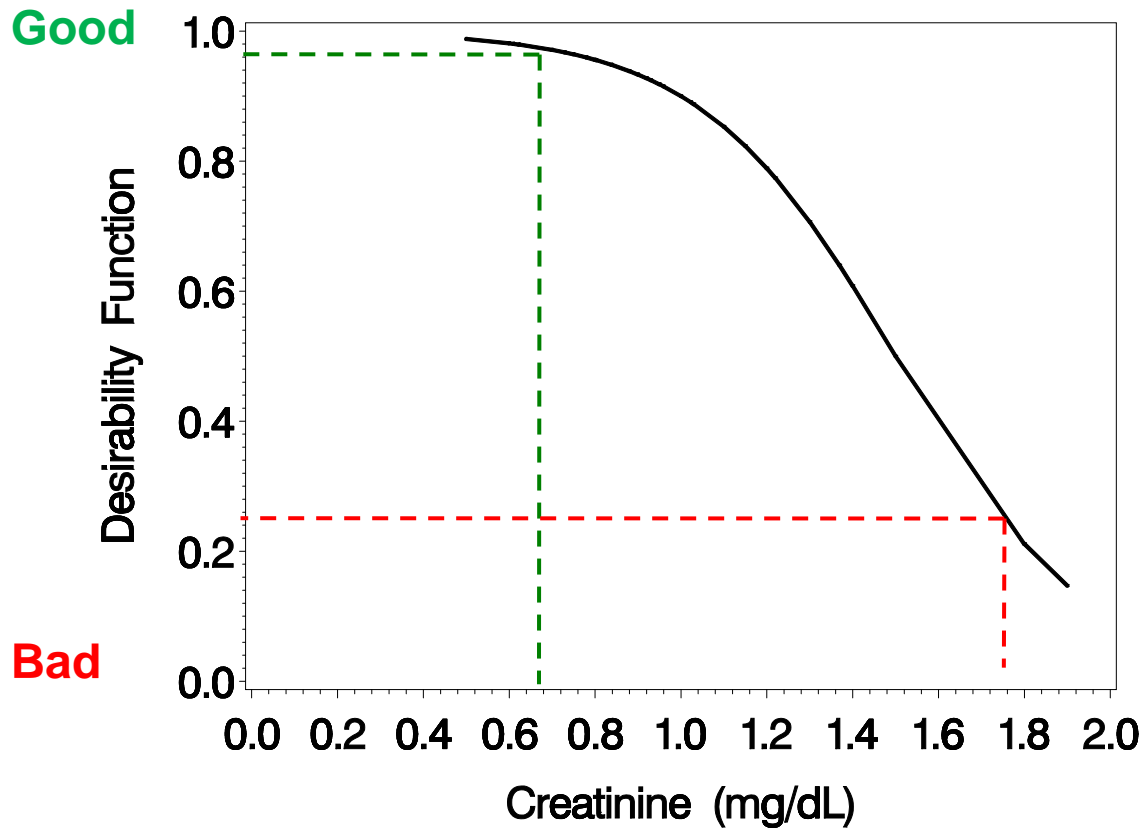
Discrimination	Q-FP	AUROC	95% CI	Sensitivity	Specificity
F0 vs F1-4	NoStr	0.87	0.75-0.99	0.84	0.78
	StrEccentricity	0.87	0.75-0.99	0.84	0.78
	StrSolidity	0.87	0.75-0.99	0.84	0.78
	StrLengthV	0.88	0.75-1.00	0.86	0.78
F0,1 vs F2-4	NoStr	0.81	0.68-0.93	0.81	0.71
	StrEccentricity	0.81	0.68-0.93	0.81	0.71
	StrSolidity	0.81	0.69-0.93	0.81	0.71
	StrLengthV	0.83	0.71-0.95	0.68	0.86
F0-2 vs F3,4	NoStr	0.89	0.79-0.98	0.90	0.82
	StrEccentricity	0.89	0.79-0.98	0.90	0.82
	StrSolidity	0.89	0.79-0.98	0.90	0.82
	StrLengthV	0.86	0.76-0.97	0.84	0.76
F0-3 vs F4	NoStr	0.92	0.84-1.00	0.90	0.81
	StrEccentricity	0.92	0.84-1.00	0.90	0.81
	StrSolidity	0.92	0.84-1.00	0.90	0.81
	StrLengthV	0.91	0.82-1.00	0.90	0.83



# Distribution of qFPs capture differences between fibrosis stages with fidelity



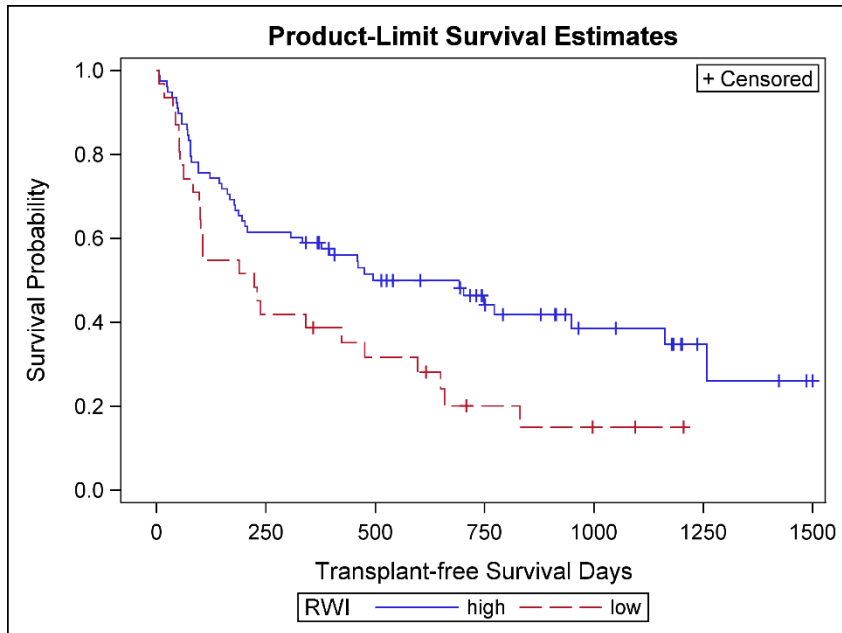
# Use of desirability functions in complex medical sciences



$$DF = (d_1 \times d_2 \times \dots \times d_k)^{1/k}$$

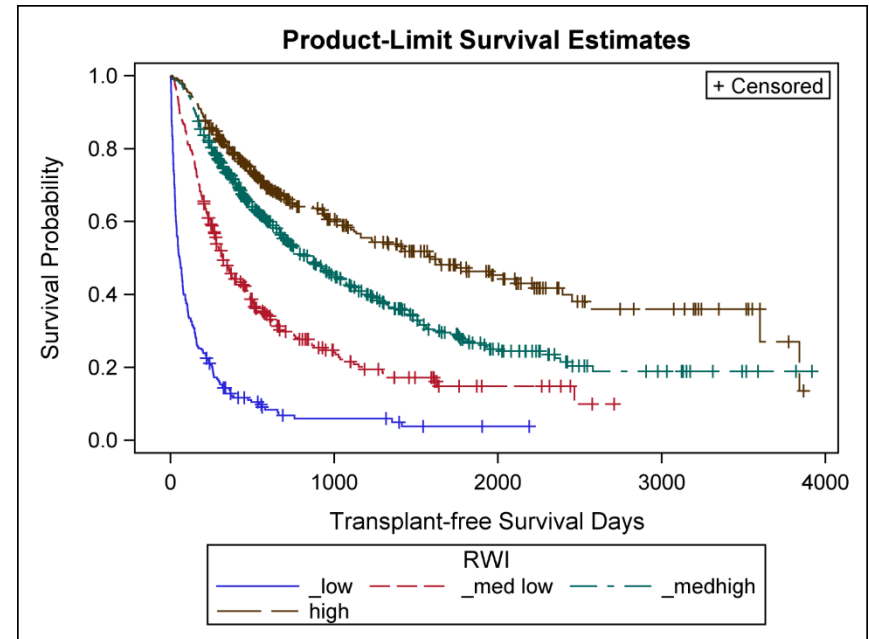
# Desirability functions provide a clear method to predict mortality in patients with cirrhosis

NASTRA population



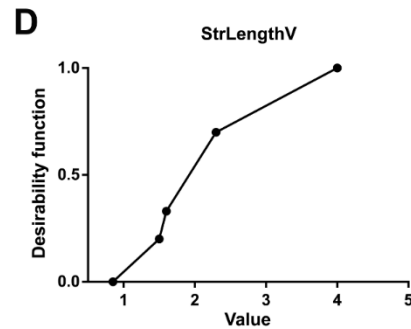
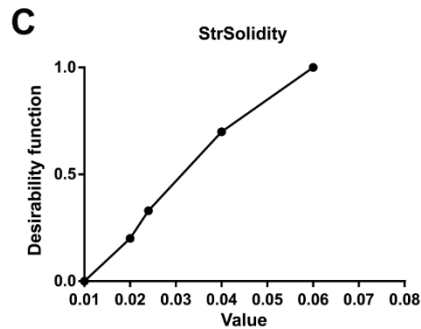
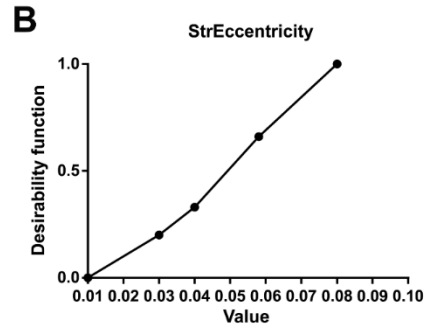
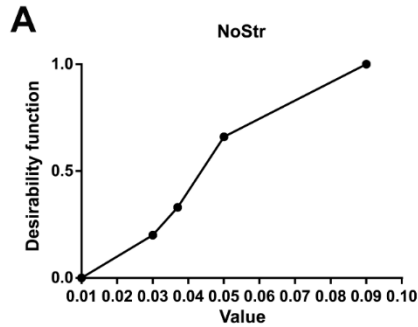
N=105

Veterans Hospital population

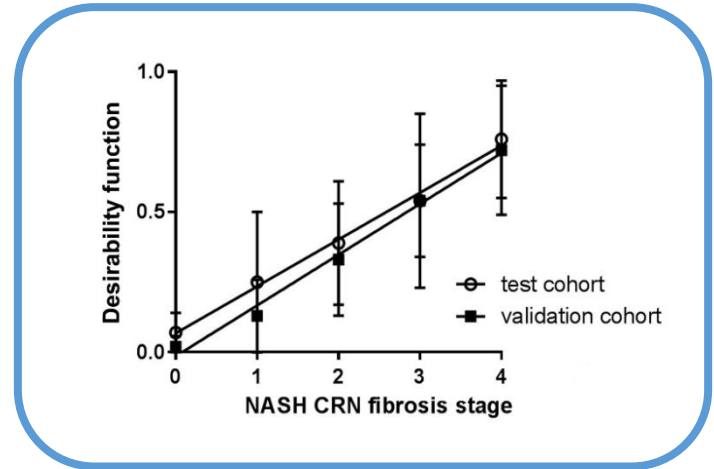


N= 1332

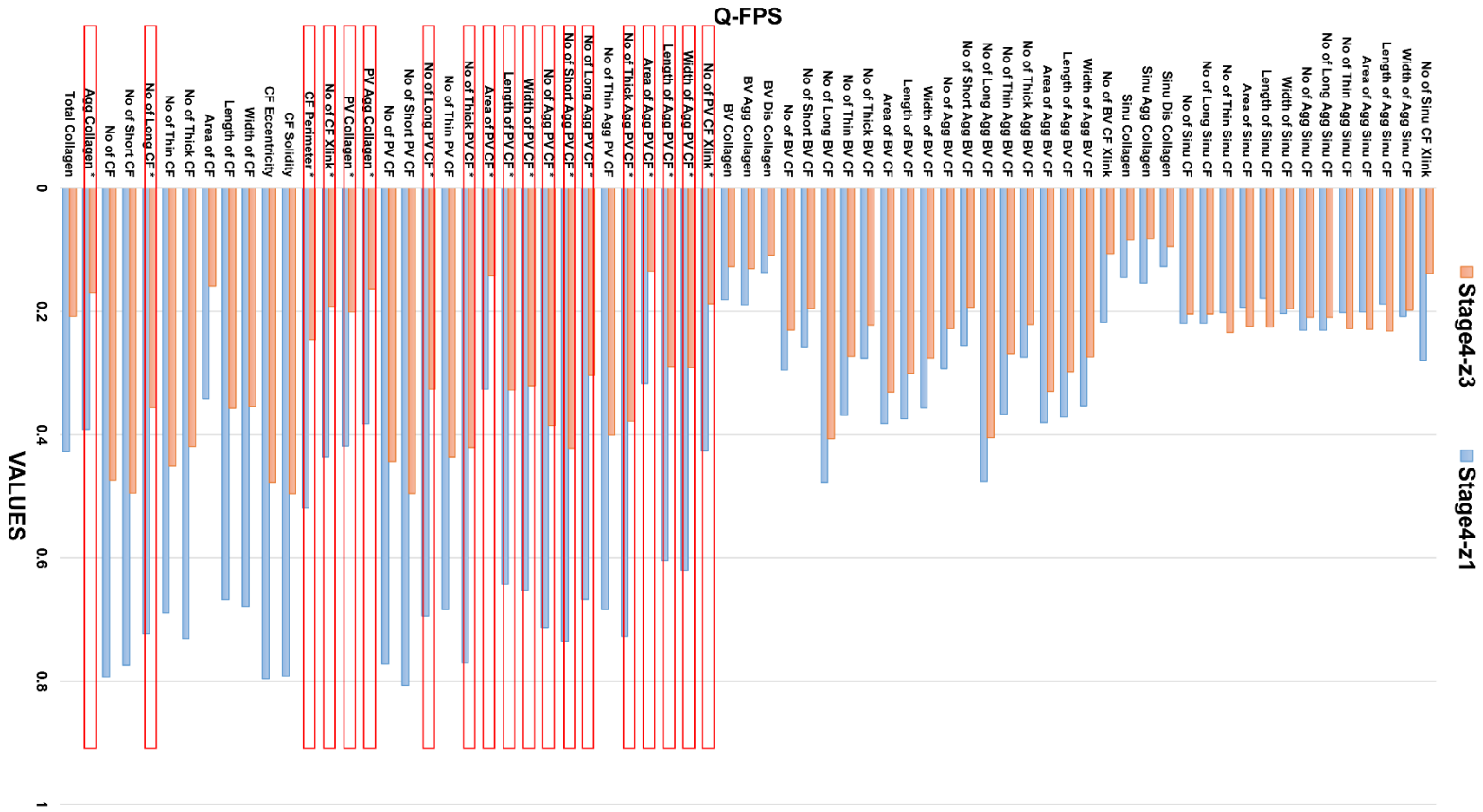
# Establish Desirability function for dynamic scaling of NASH fibrosis



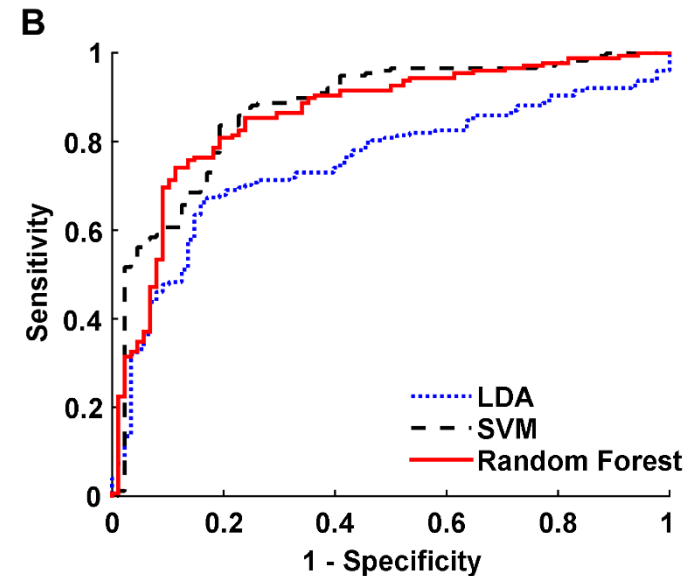
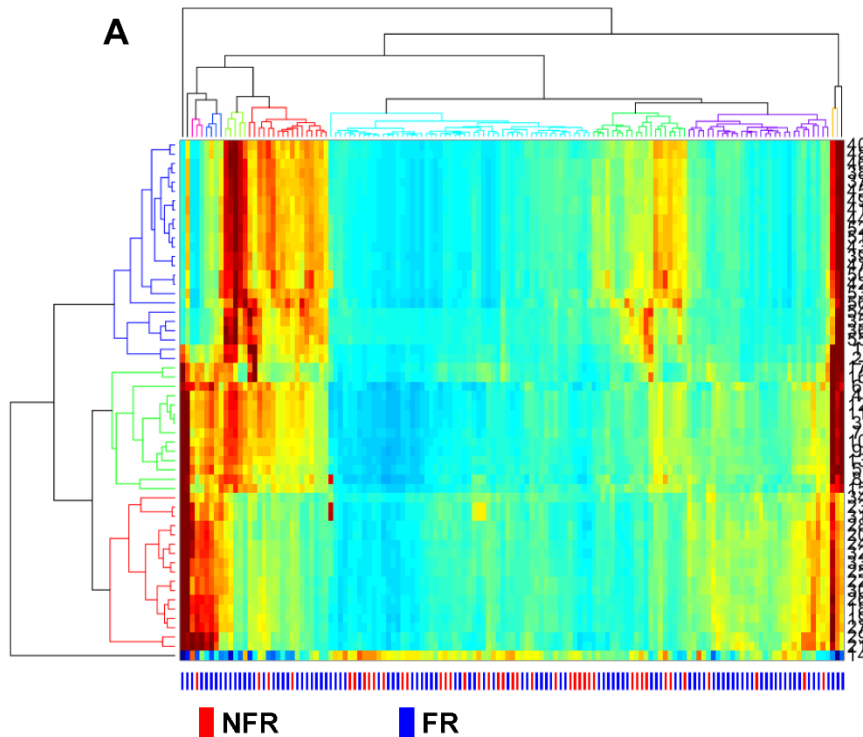
$$\text{q-FP Desirability Index} = (d_1 \times d_2 \times \dots \times d_n)^{1/4}$$



# Differential changes in portal predominant fibrosis vs central-central fibrosis in stage 4 NASH

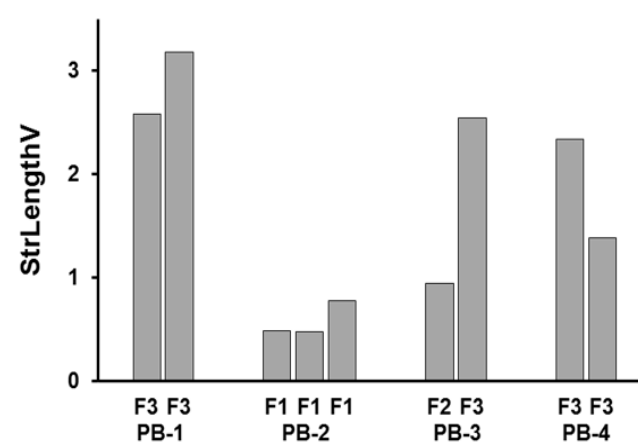
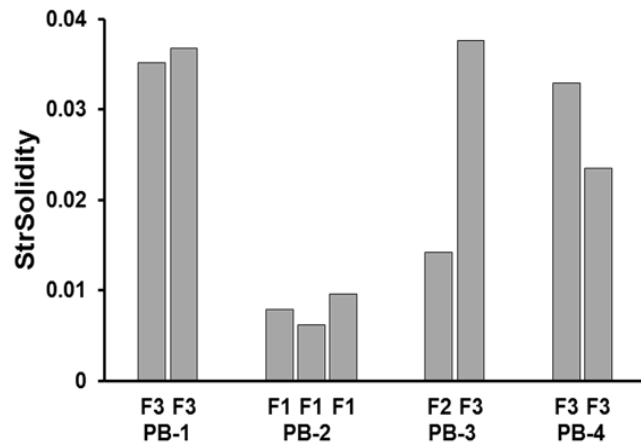
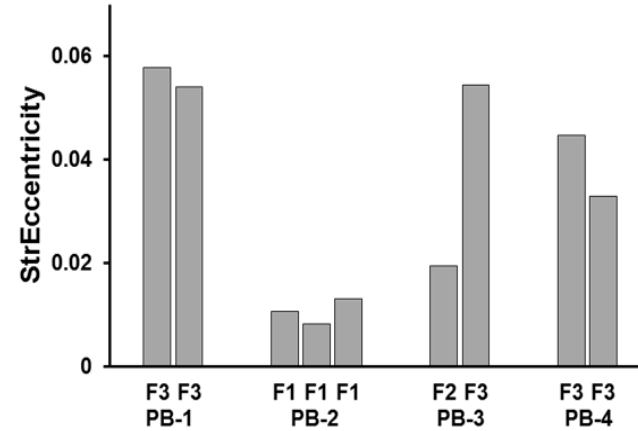
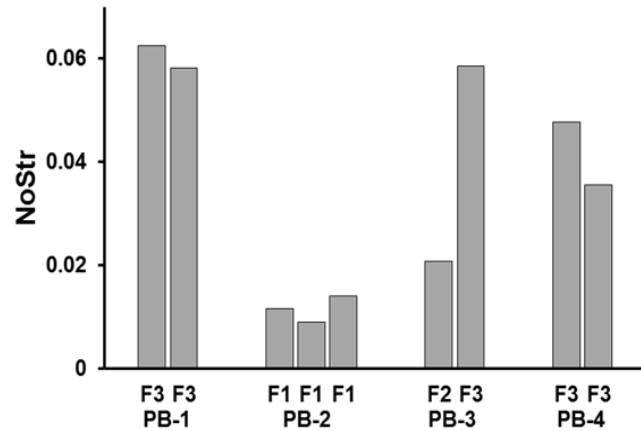


# Baseline Fibrosis Signature That Could Be More Reversible following antiviral therapy for HBV

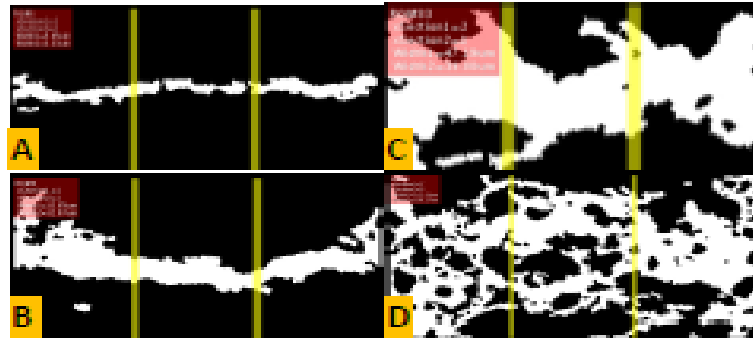


Sun J, et al. Hepatology 2014;59:1283. Xu S & Wang Y, et al. J Hepatol 2014;61:260. Wang Y, et al. Sci Rep 2017.

# Changes in qFP over time

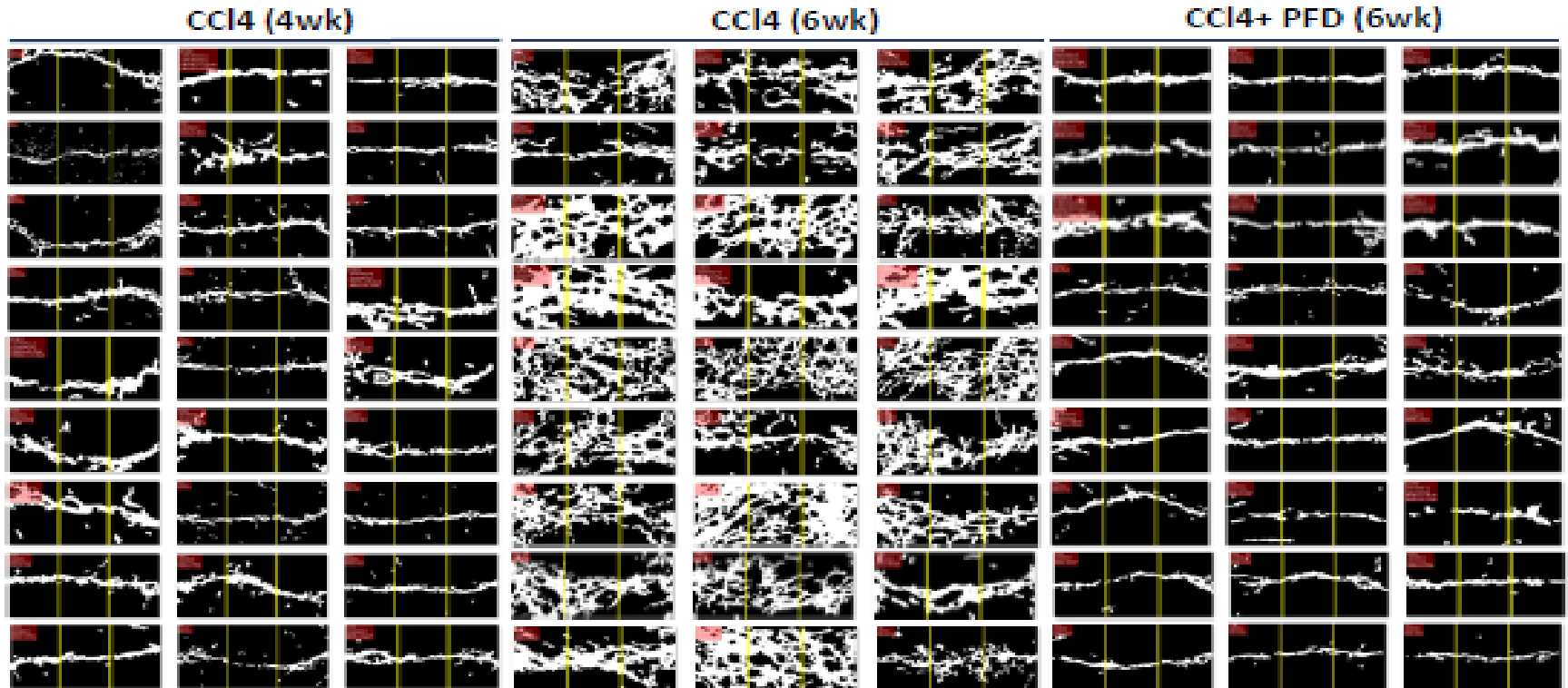


**B. Examples of various septa types and their respective value gradient**



	Septal Bridge Characteristics				
	Width	Branches	Segment Length	Collagen Reticulation Index	Collagen Area
	at 1/3 & 2/3 intersection				
A					
B					
C					
D					

**C. Fibrous septa development in CCl4-treated mice with or without Pirfenidone (PFD).**





# Future directions

- Cross-validation across cohorts
- Assessment of changes over time and early prediction of cirrhosis
- Assessment of changes vs clinical outcomes

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